STUDIES OF VASCULAR ENDOTHELIAL CELL SURFACE ANTIGENS RELEVANT TO THE ALLOIMMUNE RESPONSE

A Thesis submitted to the University of Adelaide
as a requirement for the degree of
Doctor of Philosophy

by

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ABSTRACT

This thesis examines the role of vascular endothelial cells in inflammation, with particular reference to their participation in the immune response directed against renal allografts. The studies focus on the cell surface molecules expressed by the vascular endothelium that are involved in the interactions with host effector cells and with the supporting extracellular matrix. The main text is divided into four sections. The first is a brief introduction to vascular endothelial cell biology and to some of the methods used for studying the endothelium in vitro. The second section examines the potential of vascular endothelial cells to stimulate an alloimmune response. The studies in this part document the expression of HLA class I and class II molecules on the endothelium and associated tissues in two clinical examples of an inflammatory response: (1) renal allograft rejection, and (2) vasculitis involving the peripheral nervous system. The third section concerns the cell surface molecules that mediate the interactions between the vascular endothelial cells and the extracellular matrix. In these studies, a monoclonal antibody (QE.2E5), which recognises a family of these receptors (the β_1 integrins), was produced and characterised. In addition, other monoclonal antibodies were used to study the cellular and tissue distribution of these receptors, as well as their function on vascular endothelial cells. The final section deals with the receptors that mediate the interactions between the vascular endothelium and peripheral blood leucocytes during both physiological trafficking and in inflammation. The initial studies examined the adhesion of leucocytes to cultured human umbilical vein endothelial cells (HUVEC). A monoclonal antibody (QE2.1B4), which recognises intercellular adhesion molecule-1 (ICAM-1), was produced and characterised, and used to analyse the adhesion of T lymphocytes to HUVEC. The final studies look at the expression of ICAM-1 during renal allograft rejection and in vasculitis involving the peripheral nervous system.

DECLARATION

I declare that

(a) this thesis contains no material which has been accepted for the award of any other degree or diploma in any University and that, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text; and

(b) I consent to this thesis being made available for photocopying and loan if applicable if accepted for the award of the degree.

RANDALL J. FAULL

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(7) the many investigators who supplied antibodies used in these studies
(8) my wife Diana for assistance with the typing

DEDICATION

I wish to dedicate this thesis to three people.

(1) my father, James Francis Faull, who unfortunately failed by 3 months to survive to see this thesis completed. As a prolific author, he would have been proud to receive a copy of his eldest son's thesis.

(2) my daughter Rachel Louise, who successfully battled against great odds to be alive when this thesis was completed.

(3) my wife Diana, whose love and strength kept me going while all around threatened to crumble.

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SUMMARY OF THESIS

This thesis examines the role of vascular endothelial cells in inflammation, with particular reference to their participation in the immune response directed against a vascularised allograft (kidney). The studies focus on the cell surface molecules expressed by the vascular endothelium that are involved in the interactions with host effector cells and with the supporting tissues.

The main text is divided into four sections. The first is a brief introduction to vascular endothelial cells, which includes a discussion of the methods used for studying them in vitro, their role in coagulation and thrombosis, and a list of some of their metabolic and synthetic functions. The subsequent three sections detail the studies performed for this thesis, and each commences with a literature review of the cell surface molecules that are the subject of that section.

The second section examines the potential of vascular endothelial cells to stimulate an alloimmune response. The literature review at the commencement of this section deals with the polymorphic antigens expressed by vascular endothelial cells, and discusses the evidence concerning their ability to function as fully competent antigen presenting cells. The studies performed for this part first examine by an indirect immunoperoxidase staining technique the distribution and level of expression of MHC antigens in the normal human kidney compared with rejecting renal allografts, and show that both HLA class I and II antigens are increased during rejection on the vascular endothelium, and appear de novo on the tubules. It is postulated that this increase adds to the number of targets available for host effector cells as well as facilitating the antigen-presenting function of the endothelium. In parallel studies, the expression of MHC antigens in normal muscle and nerve was compared with that in autoimmune vasculitis involving those tissues. In this example of an inflammatory response against self rather than an allograft, the endothelial cells again express increased MHC antigens, which is likely to improve their antigen-presenting function.

The third section concerns the interactions between the vascular endothelial cells and the surrounding extracellular matrix, and specifically addresses the cell surface molecules that mediate the cell-matrix adhesion. The literature review briefly discusses the components of the extracellular matrix, followed by a detailed discussion of a family of heterodimeric adhesion molecules known as the integrins. The two subgroups of the integrins that are involved in cell-matrix adhesion (the β_1 integrins or very late antigens, and the

β_3 integrins or cytoadhesins) are reviewed in this section. The results for this part first detail the production and characterisation of a murine monoclonal antibody (QE.2E5) that recognises an epitope on the integrin β_1 chain, and provide evidence that there are distinct subgroups of β_1 chains identified by different monoclonal antibodies. This is followed by studies of the expression of the six β_1 integrins by cultured vascular endothelial cells and in normal spleen and kidney, as well as changes in their expression during renal allograft rejection. The functional role of two of these integrins ($\alpha_2\beta_1$ and $\alpha_5\beta_1$) on cultured vascular endothelial cells was then examined with two α chain-specific monoclonal antibodies, including one (PHM2) that was characterised as an anti-integrin antibody during the course of these studies. Finally, evidence is presented that cultured vascular endothelial cells also express the β_4 integrin chain in association with the α_6 chain.

Section four deals with the interactions between the vascular endothelium and peripheral blood leucocytes that are important during both physiological trafficking and in inflammation. The literature review discusses the mechanisms of leucocyte adherence to the endothelium followed by extravasation, and introduces the relevant leucocyte and endothelial adhesion molecules (including the β_2 integrins or leucocyte adhesion molecules). Some of the discussion concerns neutrophil and monocyte interactions with the endothelium, but mainly deals with lymphocyte-endothelial binding as this is most relevant to the alloimmune response. The studies described first deal with the work involved in setting up an assay to quantify the adhesion of peripheral blood leucocytes and related cell lines to cultured endothelial cells. This assay can be used to examine the effects of stimulating the adherent cells and/or the endothelial cells. The results obtained with neutrophils and the myeloid cell line HL60 are presented and compared with those reported in similar studies in the literature, followed by the results and relevant literature review of the adhesion T lymphocytes and lymphoid cell lines (Mann and Jurkat). As a direct consequence of these studies, work was undertaken to produce monoclonal antibodies directed against endothelial cell adhesion molecules that mediate T lymphocyte adhesion to activated endothelium, which is an in vitro correlate of lymphocyte-endothelial adhesion during inflammation. A murine monoclonal antibody (QE2.1B4) that recognises the intercellular adhesion molecule-1 (ICAM-1) was produced and characterised, and used to further examine the molecular mechanisms of T lymphocyte adhesion to activated endothelium. Finally, the expression of ICAM-1 was examined in normal kidney, muscle, and nerve, and compared with that in

renal allograft rejection and vasculitis involving muscles and nerves. The endothelial cell expression of ICAM-1 was increased in both types of immune response, and like the changes observed in MHC expression, would be expected to increase their antigen-presenting ability and accessibility as targets. In addition, the de novo expression of ICAM-1 by the renal tubules during allograft rejection was documented during these studies.

MATERIALS AND METHODS

Contents

This chapter describes the materials and methods used in the studies for this thesis. Additional specific information is provided for certain experiments where they are described in the text.

1. Antibodies used in this thesis

A large number of antibodies were obtained from various sources, and they will be listed in this section. In the text of the thesis, the antibodies will be referred to by their designated names and the appropriate reference given.

W6/32: murine IgG_{2a} monoclonal antibody directed against a monomorphic determinant on HLA class I (A, B, and C) molecules. The cell line was obtained from the American Type Culture Collection (ATCC), Maryland USA.

RM7.30: murine IgG_3 monoclonal antibody directed against a monomorphic determinant on HLA class I molecules. The cell line was obtained from the laboratory of Dr. A. d'Apice, at that time at the Department of Nephrology, Royal Melbourne Hospital, Melbourne, Victoria.

RM5.112: murine IgG_{2a} monoclonal antibody directed against a monomorphic determinant on HLA class II (DR, DQ, and DP) molecules. The cell line was obtained from Dr. A. d'Apice, Royal Melbourne Hospital.

L243: murine IgG_{2a} monoclonal antibody directed against a determinant on HLA DR molecules. The cell line was obtained from the ATCC.

Genox 3.53: murine IgG_1 monoclonal antibody directed against HLA DQ1 molecules. The cell line was obtained from the ATCC.

B7/21: murine IgG_3 monoclonal antibody directed against HLA DP molecules. The cell line was obtained from Dr. M. Honeyman, NSW Red Cross Blood Transfusion Service, Sydney, NSW.

A-1A5: murine IgG_{2b} monoclonal antibody directed against the integrin β_1 chain. Ascites was obtained from Dr. M. Hemler, Dana-Farber Cancer Institute, Boston, Massachusetts.

TS2/7: murine IgG_1 monoclonal antibody directed against the α chain of the VLA-1 molecule. Ascites was obtained from Dr. M. Hemler, Dana-Farber Cancer Institute, Boston, Massachusetts.

RMAC11: murine IgG_{2a} monoclonal antibody directed against VLA-2. The cell line was obtained from Dr. A. d'Apice, Royal Melbourne Hospital, Melbourne.

AK7: murine IgG_1 monoclonal antibody directed against VLA-2. Ascites was obtained from Dr. M. Berndt, Department of Medicine, Westmead Hospital, Westmead, Sydney, New South Wales.

J143: murine IgG_1 monoclonal antibody directed against VLA-3. Ascites was obtained from Dr. A.P. Albino, Memorial Sloan-Kettering Cancer Center, New York, New York.

B-5G10: murine IgG_1 monoclonal antibody directed against VLA-4. Ascites was obtained from Dr. M. Hemler, Dana-Farber Cancer Institute, Boston, Massachusetts.

B1E5: rat IgG monoclonal antibody directed against VLA-5. Overgrown tissue culture supernatant was obtained from Dr. C. Damsky, University of California, San Francisco, California.

GoH3: rat IgG monoclonal antibody directed against VLA-6. Overgrown tissue culture supernatant was obtained from Dr. A. Sonnenberg, Netherlands Red Cross Blood Transfusion Service, Amsterdam.

PHM2: murine IgG_1 monoclonal antibody that recognises a determinant expressed by human monocytes, platelets and endothelium. Ascites obtained from Prof. R. Atkins, Prince Henry's Hospital, Melbourne, Victoria.

Goat 172: polyclonal goat antiserum directed against the integrin β_1 chain. Serum obtained from Dr. M. Ginsberg, Scripps Clinic and Research Foundation, La Jolla, California.

TS1/22: murine IgG_1 monoclonal antibody directed against the lymphocyte function-associated antigen-1 (LFA-1). The cell line was obtained from the ATCC.

25E11: murine IgG_{2a} monoclonal antibody directed against platelet glycoprotein IIb/IIIa. Ascites obtained from Prof. G. Burns, at that time at the Division of Human Immunology, Institute of Medical and Veterinary Science, Adelaide, South Australia.

Antibody 15: murine IgG_1 monoclonal antibody directed against the integrin β_3 chain. Ascites obtained from Dr. M. Ginsberg, Scripps Clinic and Research Foundation, La Jolla, California.

439-9B: rat IgG monoclonal antibody directed against the integrin β_4 chain. Ascites obtained from Dr. S.J. Kennel, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

S3-41: murine IgG_1 monoclonal antibody directed against the integrin β_4 chain. Ascites obtained from Dr. V. Quaranta, Scripps Clinic and Research Foundation, La Jolla, California.

TS2/18: murine IgG1 monoclonal antibody directed against CD2. The cell line was obtained from the ATCC.

TS2/9: murine IgG_1 monoclonal antibody directed against lymphocyte function-associated antigen-3 (LFA-3). The cell line was obtained from the ATCC.

WEHI-CAM-1: murine IgG_1 monoclonal antibody directed against the intercellular adhesion molecule-1 (ICAM-1). Ascites obtained from Dr. A. Boyd, Walter and Eliza Hall Institute, Melbourne, Victoria.

1.2B6: murine IgG_1 monoclonal antibody directed against endothelial-leucocyte adhesion molecule-1 (ELAM-1). Overgrown tissue culture supernatant obtained from Dr. D. Haskard, Guy's Hospital, London, United Kingdom.

QE7.3E8: murine IgG_1 monoclonal antibody directed against the human CD44 molecule, produced in our laboratory.

MEL-14: rat IgG monoclonal antibody directed against the murine lymphocyte homing receptor. The cell line was obtained from the ATCC.

P3X63Ag8: murine IgG_1 monoclonal antibody of unknown specificity that does not bind to human tissues, used as a negative control. The cell line was obtained from the ATCC.

1D4.5: murine IgG2a monoclonal antibody directed against the 11RX strain of salmonella enteritidis, used as a negative control. Supernatant and ascites obtained from Dr. Leonie Ashman, Department of Microbiology and Immunology, University of Adelaide, Adelaide, South Australia.

2. Cell culture and preparation of cells

Cell culture media

The standard medium used in this thesis was RPMI 1640 (Flow Laboratories, McLean, Virginia, USA) with 100mM sodium pyruvate, 0.2% sodium bicarbonate, 10mM Hepes buffer, 160 mg/litre gentamycin, and 2mM glutamine. The cell lines used in this thesis were routinely maintained in RPMI 1640 supplemented with 10% foetal calf serum (FCS). Hybridoma cell lines were maintained in RPMI 1640 supplemented with 15% FCS. Human umbilical vein endothelial cells were cultured in RPMI 1640 supplemented with 20% FCS, plus 0.3 mg/ml endothelial cell growth factor (ECGF) (Sigma, St. Louis, MO) and 30 Units/ml porcine mucous heparin sodium (Fisons, Thornleigh, NSW). All cells were cultured at 37°C in 5% CO_2.

Purification of human peripheral blood mononuclear cells (Boyum, 1968)

Blood samples from normal volunteers were placed in heparinised tubes and centrifuged at 600g for 10 minutes, followed by removal of approximately two-thirds of the revealed plasma to deplete the sample of platelets. The remaining blood was diluted 1 in 3 in 0.9% saline and 18 ml aliquots were distributed into V-bottomed 30 ml plastic tubes (Disposable Products, Adelaide). The diluted blood was then underlayed with 4 ml of Lymphoprep (Nycomed, Oslo, Norway) and centrifuged at 1500g for 20 minutes. The mononuclear cells at the Lymphoprep-plasma interface were carefully removed by pipetting into V-bottomed 10 ml plastic tubes, diluted 1 in 3 in 0.9% saline, and pelleted at 2000g for 5 minutes. The pellet was resuspended in 10 ml of 0.9% saline and the cell numbers determined by counting using a Neubauer haematocytometer.

Purification of monocytes from human peripheral blood

Mononuclear cells were purified from donor blood (typically 30 ml) by the above method and resuspended in 5ml of RPMI 1640 supplemented with 20% heat-inactivated newborn bovine serum (HINBS). Plastic 9 cm petri dishes (Johns, Clayton, Victoria) were coated with heat-inactivated human AB serum for 30 minutes at 37°C, followed by its removal by pipetting. The mononuclear cell suspension was poured onto the petri dish and incubated at 37°C for 30 minutes. Under these conditions the monocytes will adhere to the dish and the lymphocytes will remain in suspension. The adherent monocytes are readily visible by phase contrast microscopy as dark cells adherent and spreading on the plate. Following incubation, the non-adherent cells are washed off by pipetting the medium into 10 ml tubes and further washing with RPMI 1640 (supplemented with 5% HINBS) to a volume of 20 ml. These non-adherent cells were kept if purified T and/or B lymphocytes were required. The adherent cells were removed from the plate by incubation in 18 ml of 0.4% ethylenediamenetetraacetic acid (EDTA) at 37°C for 10 minutes. The detached cells were washed by pipetting into 10 ml plastic tubes and pelleted by centrifuging at 2000g for 5 minutes. The cells were resuspended in RPMI 1640 with 5% HINBS and counted using a Neubauer haematocytometer. The purity

Purification of T lymphocytes from human peripheral blood
(Danilovs et al, 1980)

Sixty milligrams of nylon wool fibre (Fenwal Laboratories, Illinois, USA) was teased out and packed loosely into a 12 cm plastic drinking straw (which had been heat-sealed at one end) so that 1 cm at each end was left empty. A small drainage hole was cut into at the sealed end, and the packed straw was washed with plain RPMI 1640. The straw containing the saturated nylon wool fibre was then incubated at 37°C for 30 minutes. The non-adherent cells stored after the purification of monocytes were pelleted at 2000g for 5 minutes, and resuspended in 0.5 ml of RPMI 1640 plus 5% HINBS. The cell suspension was loaded onto the warmed straw which was then incubated again for 30 minutes at 37°C. Following incubation, the non-adherent T lymphocytes were washed out of the straw with 20 ml of RPMI 1640 plus 5% HINBS and pelleted at 2000g for 5 minutes. The purified T lymphocytes were resuspended in 2 ml of RPMI 1640 plus 5% HINBS and counted using a Neubauer haematocytometer. Typically 15 - 20 x 10^6 T lymphocytes were obtained from 30 ml of peripheral blood.

Purification of neutrophils from human peripheral blood

Twenty ml of donor blood was taken into a 30 ml syringe containing 4 ml of 4.5% EDTA in distilled water (pH 7.4) as anticoagulant. The blood was mixed with dextran 150 (6% in 0.9% saline) 1:5 vv, and allowed to sediment for 45 minutes at 37°C. The plasma was then carefully removed to avoid erythrocyte contamination, placed in a 50 ml plastic centrifuge tube (Corning, New York, USA), underlayed with 6 ml of Lymphoprep and centrifuged at 1500g for 20 minutes. The supernatant was carefully removed by pipetting, leaving the pellet containing neutrophils and contaminating erythrocytes. The erythrocytes were lysed by suspending the pellet in 10 ml of 0.2% saline for 25 seconds, and then adding 10 ml of 1.6% saline, followed by centrifuging at 1000g for 7 minutes. The lysis step was repeated if any erythrocytes remained intact. The purified neutrophils were resuspended in medium and counted using a Neubauer haematocytometer. Typically 30 - 40 x 10^6 neutrophils were obtained from 20 ml of blood.

Purification of platelets from human peripheral blood

Thirty ml of donor blood was anticoagulated with EDTA (4.5% in distilled water, pH 7.4, 2 ml per 10 ml of blood), and centrifuged at 600g for 10 minutes. The platelet-rich plasma was carefully removed by pipetting to avoid erythrocyte contamination, placed in a 10 ml plastic tube, and centrifuged at 2000g for 20 minutes at 4°C (to avoid platelet clumping). The pellet was resuspended in cold medium and the cells counted using a Neubauer haematocytometer. Typically, about 10 x 10^8 platelets were obtained from 30 ml of blood.

Isolation and culture of human umbilical vein endothelial cells (HUVEC)

HUVEC were isolated and cultured according to the technique described by Jaffe et al (1973a), with minor modifications. They were routinely cultured in tissue culture flasks which had been coated with sterile 0.1% gelatin.

Fresh human umbilical cords were obtained from the labour ward of The Queen Elizabeth Hospital, and stored in sterile Hank's balanced salt solution at 4°C until use (preferably within 24 hours of delivery). The cords were warmed up to 37°C at the time of harvesting. The cord was trimmed and the umbilical vein at one end cannulated with a sheethed 21g winged infusion set (Terumo, Melbourne, Australia). This was gently clamped in place with a plastic clamp, and the lumen of the vein washed thoroughly but gently by perfusion with 30 - 50 ml of sterile Hank's. The bottom end of the cord was clamped with another plastic clamp and the vein filled with 5 - 10 ml of warmed 0.1% collagenase (Sigma) in sterile Hank's. The clamped cord filled with collagenase was incubated in warm sterile Hank's at 37°C in a water bath for 15 minutes. The collagenase and detached cells were then washed into a 50 ml centrifuge tube by perfusing the vein with 30 ml of warmed sterile Hank's supplemented with 1% FCS (to inactivate the collagenase). The cells were pelleted by centrifugation at 1000g for 7 minutes, resuspended in 3 ml of culture medium (see above), and placed in a gelatin-coated 25 cm^2 tissue culture flask (Miles Laboratories, Naperville, Illinois, USA). After overnight incubation, any non-adherent cells were washed out with warmed sterile Hank's and a new 3 ml of medium added to the adherent cells. As a general rule, cells from individual cords were cultured in separate flasks.

The adherent cells were identified as HUVEC by their characteristic "cobblestone" pattern of growth as a monolayer which readily distinguishes them from contaminating fibroblasts and smooth muscle cells (see photograph page 30). This was confirmed by examination by electron microscopy for Weibel-Palade bodies (photograph page 30), and positive staining by indirect immunoperoxidase (see below) for factor VIII (photograph page 31).

The cells were passaged up to 75 cm^2 tissue culture flasks when the monolayer reached confluence, usually after 4 - 5 days. They were detached by either brief incubation with 0.1% EDTA/0.125% trypsin in sterile Hank's or 3 minutes incubation with 0.1% EDTA in sterile Hank's. The detached cells were washed out with sterile Hank's (plus 1% FCS to inactivate the trypsin), centrifuged at 1000g for 7 minutes, and resuspended in the appropriate volume of medium.

3. Staining techniques

Indirect Immunoperoxidase

Cell preparations or cut tissue sections were stained with monoclonal antibodies using an indirect immunoperoxidase technique. The isolated cells were cytospun onto glass slides, allowed to dry, and fixed by immersion in cold (4°C) acetone for 10 minutes. The tissue sections were in two categories. Tissue that had been previously obtained by biopsy was unfixed and had been snap frozen in liquid nitrogen. Five micron sections were cut from these specimens on a cryostat onto gelatin-coated glass slides, air-dried, and fixed in cold acetone for 10 minutes. Biopsies from renal allografts that were especially obtained for this thesis were fixed for 2 - 4 hours in periodate-lysine-paraformaldehyde (PLP) before being snap frozen in Tissue Tek II mounting medium (Miles Scientific, Illinois, USA) and stored at -70°C. Five micron sections were cut onto gelatin-coated glass slides and allowed to dry before staining.

The non-specific binding of protein was blocked by incubating the specimens with 3% normal horse serum in phosphate-buffered saline (PBS) for 10 minutes. This and all subsequent incubations were performed at room temperature in a humidified chamber. The specimens were then incubated for 45 minutes with 100 µl of the monoclonal antibody, either as neat tissue culture supernatant or as a 1 in 500 dilution of ascites in 3% normal horse serum. Following extensive washing in PBS, they were incubated for 30

minutes with 100 µl of biotinylated horse anti-mouse IgG or anti-rat IgG (depending on the source of the primary antibody) (Vector Laboratories, California, USA), diluted 1 in 200 in 3% normal horse serum. After further washing in PBS, they were incubated for 60 minutes with 100 µl of a 1:1 mixture of avidin DH and biotinylated horseradish peroxidase H (Vector Laboratories), diluted 1 in 100 in 3% normal horse serum. The specimens were again washed in PBS and exposed for 7 minutes to 0.5 mg/ml diaminobenzidine tetrahydrochloride (Sigma) and 0.01% hydrogen peroxide in 0.05M Tris-HCl buffer (pH = 7.2). A positive reaction gives a brown stain. The specimens were then counterstained with Mayer's hematoxylin and mounted under a glass cover-slip.

Indirect one-colour staining for flow cytometric analysis

HUVEC were eluted with 0.1% EDTA whereas other adherent cells were detached by vigorous pipetting of the bottom of the tissue culture flask. The cells were counted using a Neubauer haematocytometer and sufficient taken to give 5 x 10^5 cells per antibody. They were washed twice in PBS plus 1% FCS plus 0.02M sodium azide for 5 minutes at 600g at 4°C. All subsequent washes in the procedure were performed in the same manner, and the cells were resuspended in between by gently tapping the tube.

After washing, the cells were resuspended in the washing solution plus 10% normal rabbit serum at a volume of 100 µl per antibody for 10 minutes on ice. Following aliquoting into individual tubes, the primary antibody was added (50 µl of tissue culture supernatant or 10 µl of a 1 in 500 dilution of ascites) for 20 minutes on ice. After a single wash, the secondary antibody (fluoroscein isothiocyanate-conjugated sheep anti-mouse IgG [Silenus, Victoria, Australia] or anti-rat IgG [Silenus]) was added to a final dilution of 1 in 200 for 20 minutes on ice. This and all subsequent steps were performed in the dark. The cells were fixed with 2 ml of a 1:10 dilution in distilled water of fixative and lysis solution (Becton Dickinson, San Jose, California, USA), diluted 1 in 10 in distilled water, for 12 minutes at room temperature. After two washes, the cells were resuspended in 500 µl of filtered saline and stored in the dark at 4°C until analysed on a Becton Dickinson FACScan (Becton Dickinson).

Cellular enzyme-linked immunosorbent assay (ELISA)

A cellular ELISA was used to screen fusions for antibody production against cultured HUVEC and for assessment of HUVEC expression of a variety of cell surface antigens.

HUVEC were eluted from their flasks and 2 x 10^4 cells in 100 µl of medium were added to each well of a flat-bottomed 96 well microtitre plate (Disposable Products, Adelaide, South Australia). Following overnight incubation at 37°C, the cells were adherent and had spread to completely cover the bottom of the well. Any stimulation of the HUVEC (eg with lipopolysaccharide [LPS] [Sigma] or interleukin-1 α [IL-1] [Boehringer Mannheim, Mannheim, Germany) was performed at this point, and the cells were then fixed in cold 1% glutaraldehyde in PBS for 5 minutes. The glutaraldehyde was thoroughly washed out with PBS and non-specific binding blocked with 200 µl/well of a 2% solution of normal horse and/or goat serum in PBS for 10 minutes at room temperature. The choice of serum depended on the animal source of the secondary antibody used in the assay. The blocking solution was discarded and 50 µl of the primary antibody (neat tissue culture supernatant or a 1 in 500 dilution of ascites) added to each well for 30 minutes at 37°C. The primary antibody was discarded and the wells washed twice with PBS, taking care to avoid any cross-contamination. The secondary antibody (biotinylated horse anti-mouse IgG or anti-rat IgG, and/or biotinylated goat anti-mouse IgM (all Vector Laboratories, California, USA), diluted 1 in 200 in 2% normal serum, 50 µl/well) was added for 15 minutes at 37°C, and then washed off twice with PBS. The next incubation was with 50 µl/well of a 1:1 mixture of avidin DH and biotinylated horseradish peroxidase H (Vector Laboratories), diluted 1 in 100 in PBS plus 0.1% Tween 20, for 15 minutes at 37°C. Following 5 washes in PBS, the cells were incubated with 100 µl/well of 0.01% 2,2'-azinobis(3-ethylbenzthiazoline sulfonic acid) (ABTS) (Sigma) plus 0.015% H_2O_2 in 50mM citrate buffer in PBS, pH 5.3, for 30 minutes at 37°C in the dark. A positive result was revealed by a green colour change, which was quantified on a Titertek multiskan MC (Flow Laboratories, McLean, Virginia, USA) at 414 nm.

4. Production of monoclonal antibodies (Kohler 1981)

Animals

Female Balb/c mice were used for immunisation and production of immune ascites.

Immunisation

Approximately 3 - 5 x 10^6 HUVEC were stimulated while still in their tissue culture flasks with either 100 ng/ml of LPS or 4 Units/ml of IL-1 for 4 hours at 37^{0}C. The cells were then eluted with 0.1% EDTA in sterile Hank's solution and washed 4 times in plain RPMI 1640 at 1000g for 7 minutes to remove all FCS. After resuspension in 0.5 ml of plain RPMI 1640 the cells were injected intraperitoneally into a Balb/c mouse. This procedure was performed 28 and 3 days before the fusion.

Fusion partner cell line

The murine cell line SP2/0-Ag14, which was originally produced as a sub-clone of a hybrid between a Balb/c spleen cell and the myeloma cell line X63-Ag8, was used as the partner for fusions. These cells are resistant to 20 micrograms/ml of 8-azaguanine but die in HAT-supplemented medium, and do not synthesise any immunoglobulin chains (Schulman et al,1978)

Feeder cells

Thymocytes, aseptically isolated from the thymus' of young Balb/c mice, were seeded in 50 microlitre aliquots at a concentration of 4 x 10^5 cells/well into the central 60 wells of 4 flat-bottomed 96 well microtitre plates the day before the fusion, and incubated overnight at 37^{0}C.

Culture medium

Hybridomas were cultured RPMI 1640 plus 15% FCS supplemented with either HAT (1 x 10^{-4}M hypoxanthine, 4 x 10^{-7}M aminopterin, and 1.6 x 10^{-5}M

thymidine) or HT (1 x 10^{-4}M hypoxanthine and 1.6 x 10^{-5}M thymidine). These supplements were made up as 100x concentration and aliquots stored at -70°C until used.

Cell fusion

The immunised mouse was killed by cervical dislocation and the spleen aseptically removed. A single cell suspension was produced by teasing the spleen through a sterile sieve in plain RPMI 1640, and washed 3 times. A total of 1 x 10^8 spleen cells were mixed with 1 x 10^7 SP2/0-Ag14 cells and centrifuged at 600g for 7 minutes. The supernatant was carefully completely removed by aspiration, and the cell pellet disrupted by "flicking". The tube was placed in a water bath at 37°C and 0.7 ml of prewarmed 50% polyethylene glycol 1500 (Merck, Munich, Germany) was added over 1 minute with gentle stirring. The mixture was stirred for a further minute and 10 ml of plain RPMI 1640 at 37°C added over the next 5 minutes with continual stirring. A small sample of the mixture was observed microscopically to determine if fusion had taken place, and the remainder was centrifuged at 600g for 7 minutes. The pellet was resuspended in 15 ml of RPMI 1640 plus 15% FCS, and 50 microlitre aliquots were placed in the wells which had previously been seeded with feeder cells. Plain medium was used to fill the outside wells of the plates, and they were incubated overnight at 37°C. The next day 100 µl of 2 x HAT medium was added to each well, and from then on the wells were observed daily for growth of hybridoma colonies.

Screening of fusions

At 7 to 14 days after fusion supernatant was taken from the wells containing hybridoma colonies and assayed by cellular ELISA against cultured HUVEC for the presence of specific antibodies. The supernatants were screened in parallel against both unstimulated and LPS- or IL-1-stimulated HUVEC (depending on the stimulant used on the immunising cells) to detect antibody against antigens more highly expressed by activated HUVEC. Hybridomas of interest were then expanded, cloned, and the cells frozen and supernatant kept for further testing. The culture medium was changed to HT supplemented after 10 to 14 days, and several days later both the hypoxanthine and thymidine were omitted.

Cloning of hybridomas

Hybridomas were cloned by limiting dilution in flat-bottomed 96 well microtitre trays containing mouse thymocytes as feeder cells. Thirty six wells were seeded with viable hybridoma cells at a concentration of 5 cells/well, 36 wells at 1 cell/well, and 24 wells at 0.5 cells/well. After 3 to 4 days of culture, the number of colonies in each well were counted, and when they were of sufficient size (day 7 to 10 usually), the supernatants were assayed again for antibody against HUVEC by the cellular ELISA. A colony from a single colony well was grown up and recloned, and the hybridoma was considered to be monoclonal when all wells containing hybridoma cells produced supernatants containing anti-HUVEC antibody. The cells of a positive well containing a single colony were then expanded for cryopreservation and production of tissue culture supernatant and immune ascites.

Cryopreservation of cells

Approximately 10 ml of well-grown culture was centrifuged at 600g for 7 minutes and the pellet resuspended in 1 ml of RPMI 1640 plus 15% FCS. An equal volume of 20% dimethyl sulphoxide (Ajax chemicals, NSW, Australia) in the same medium was added dropwise to the cell suspension at 4^{0}C while the tube was shaken. The mixture was placed in cryotubes (Nunc, Denmark) and frozen at a controlled rate in a Handi-Freeze freezing tray (Union Carbide). The ampoules were stored in the liquid phase of liquid nitrogen in a Union Carbide 35VHC liquid nitrogen tank until used.

Preparation of immune ascites

Balb/c mice were injected twice, one week apart, with 0.5 ml of Pristane (2,6,10,14-tetramethylpentadecane, Aldrich Chemical Company, Wisconsin, USA). Three days after the second injection, they were injected intraperitoneally with 2 x 10^{6} washed hybridoma cells suspended in plain RPMI. The mouse was inspected daily and when it developed marked abdominal distension (usually after 6 to 10 days), the mouse was sacrificed and the ascites aspirated by incising the peritoneal membrane. The sample was centrifuged and the ascites aspirated and stored at -70^{0}C until required.

Isotyping of antibodies

The isotypes of the monoclonal antibodies produced as a part of this thesis were determined using a Serotec MMT RC1 isotyping kit (Serotec, Oxford, England).

5. Methods for immunoprecipitation studies

Lactoperoxidase catalysed cell surface iodination

Reagents:

(1) high buffer PBS - NaCl 3.85 gm, Na_2HPO_4 1.135 gm, and KH_2PO_4 0.27 gm made up to 500 ml with distilled water.
(2) 1/1000, 1/3000, 1/9000, and 1/27000 dilutions of 30% H_2O_2 in distilled water.
(3) lactoperoxidase (Calbiochem, La Jolla, California, USA), 0.2 mg/ml in high buffer PBS.
(4) ^{125}I as sodium iodide (New England Nuclear Products, Boston, USA).
(5) PBS plus 0.02% sodium azide and 1mM potassium iodide at 4°C.

Method:

The cells to be labelled were isolated and washed 3 times in high buffer PBS to remove extraneous protein. HUVEC were detached from the tissue culture flasks by 0.1% EDTA alone. The number of cells varied from about 1 x 10^7 HUVEC to 2 - 5 x 10^7 of a cell line to 1 x 10^8 platelets. They were resuspended in 200 µl of cold (4°C) high buffer PBS, and mixed with 75 µl of lactoperoxidase solution and 0.5 mCi of sodium iodide (as ^{125}I). At one minute intervals, 10 µl of increasing concentrations of H_2O_2 were successively added to the mixture. One minute after the addition of the 1/1000 dilution of H_2O_2, the cells were flooded with excess cold PBS plus sodium azide and potassium iodide, and then washed 3 times in the same solution.

Detergent solubilisation of labelled cell surface molecules

Following washing, the pellet of ^{125}I-labelled cells was resuspended in 1 ml of lysis buffer (0.5% Triton X-100 [Biorad, Richmond, California, USA] in PBS) and incubated on ice for 60 minutes. The mixture was then centrifuged at 2500g for 5 minutes to pellet the nuclei and cell debris.

Immunoprecipitation using staphylococcus aureus cells

The lysate remaining after solubilisation was precleared for 60 minutes at 4°C with 200 µl of 10% suspension of Staphylococcus aureus, Cowan Strain I cells (Pansorbin, Calbiochem) to remove proteins which bind non-specifically to staphylococcal cells. After centrifugation to remove the staphylococci, antibody (supernatant 200 µl, ascites 5 µl) and 5 µl of rabbit anti-mouse Ig or 20 µl of rabbit anti-rat Ig (Dakopatts, Denmark) were added to the lysate and incubated at 4°C overnight. Two hundred µl of the 10% suspension of Staphylococcus aureus cells plus 1 mg/ml ovalbumin was then added and incubated at 4°C for 60 minutes. The cells were then washed 3 times in SAC buffer (PBS plus 0.5% Triton X-100, 5mM potassium iodide, and 0.02% sodium azide) at 4°C, and the dried pellet stored at -70°C until required for electrophoresis.

Preparation of samples for electrophoresis

(a) Samples for one-dimensional sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

Reagents

SDS-sample buffer
- Trizma base 3.8 gm (62mM) (Sigma)
- glycerol 50 ml (10%) (Ajax Chemicals)
- SDS 11.5 gm (0.2%) (BDH Chemicals, Victoria, Australia)
- dithiothreitol 3.9 gm (50mM)(Biorad)

Distilled water to 500 ml
pH to 6.8 with concentrated HCl
Store at 4°C
Dithiothreitol is left out for non-reducing gels

Method

Resuspend sample pellets in 100 µl of sample buffer, plus one tube containing sample buffer and molecular weight standards. The tubes are then heated in a boiling water bath for 5 minutes, and the staphylococcal cells are centrifuged out. The resulting supernatants are then ready for loading into the wells of the prepared slab gel (see below).

(b) Samples for two-dimensional PAGE

Reagents

Isoelectric focussing (IEF) sample buffer

- urea 5.7 gm (9.5M) (Biorad)
- Triton X-100 0.2 ml (2%)
- pH 5 - 7 ampholines (LKB, Sweden) 0.4 ml
- pH 3.5 - 10 ampholines (LKB) 0.1 ml
- dithiothreitol 78 mg (50mM)

Distilled water to 10 ml

Stored in 200 µl aliquots at -70°C until required

Method

Resuspend pellet in 50 µl of sample buffer for 60 minutes at room temperature, and then centrifuge out cells. The resulting supernatant is ready to be loaded onto the top of a tube gel for IEF.

Preparation of gels for electrophoresis

Reagents

(1) 30% acrylamide for SDS gels

- acrylamide (Biorad) 300 gm
- bis-acrylamide (Biorad) 8.2 gm

Distilled water to 1027 ml

Filter through filter paper and store at 4°C protected from light

(2) 30% acrylamide for IEF gels

- acrylamide 14.2 gm
- bis-acrylamide 0.8 gm

Distilled water to 50 ml

Filter through filter paper and store at 4°C protected from light

(3) Lower gel buffer

- Trizma base 182 gm (1.5M)
- SDS 4 gm (0.4%)

Distilled water to 900 ml

pH to 8.8 with concentrated HCl, then make up to 1000 ml

Store at 4°C

(4) Upper gel buffer

- Trizma base 30 gm (0.5M)
- SDS 2 gm (0.4%)

Distilled water to 400 ml
pH to 6.8 with concentrated HCl, then make up to 500 ml
Store at 4°C

SDS slab gels for one dimensional gel electrophoresis

The slab gel apparatus was assembled and the lower resolving gel poured usually at least 12 hours before use. The concentration of acrylamide in the resolving gel was determined by the molecular weight of the antigens to be examined eg a 5% acrylamide gel covers the molecular weight range 70 to 200 kilodaltons (kD), and a 7.5% gel covers 40 to 150 kD. The proportion of 30% acrylamide stock solution was therefore adjusted accordingly and added to a mixture of lower gel buffer (25%) and distilled water, followed by (for a 5% gel) 80 µl of 10% ammonium persulphate (Biorad) and 40 µl of N,N,N',N' - tetramethylanediamine (TEMED) (Biorad) to induce polymerisation. The lower gel was poured, overlayed with N-butanol (Sigma), and allowed to polymerise at room temperature. This typically took about 60 minutes, but was generally left overnight.

The stacking gel mixture consisted of 2.5 ml of upper gel buffer, 5.9 ml of distilled water, 1.6 ml of 30% acrylamide, 30 µl of 10% ammonium persulphate, and 10 µl of TEMED. The N-butanol was aspirated from the lower gel, and the stacking gel poured with a teflon comb inserted to form the sample wells. The mixture was allowed to polymerise at room temperature, typically in about 30 minutes.

Tube gels for first dimension (IEF) of two-dimensional PAGE (O'Farrell 1975)

Gel mixture

- urea 2.75 gm (9.2M)
- 30% acrylamide solution 0.67 ml (4% acrylamide)
- 10% Triton X-100 (2%)
- distilled water 1 ml
- pH 5 - 7 ampholine 200 µl
- pH 3.5 - 10 ampholine 50 µl
- 10% ammonium persulphate 7 µl
- TEMED 4.5 µl

These quantities are sufficient to make 9 tube gels

Method

Tube gels were cast in 150 mm length borosilicated tubes of 1.5 mm internal diameter (Biorad), capped at the lower end with 4 layers of Parafilm (American Can Company, Connecticut, USA). The gel mixture was heated slightly to ensure dissolution of the urea and a 12 cm long gel poured into each tube using an 20 cm needle and syringe, taking care to ensure that no air bubbles were trapped in the mixture. The mixture was overlayed with distilled water and allowed to polymerise at room temperature (approximately 45 minutes). The water overlay was then "flicked" off and replaced by 20 µl of IEF sample buffer.

Electrophoresis procedure

(a) SDS-PAGE

Reagents

SDS running buffer (10 x stock)

- Trizma base 303 gm
- glycine 1440 gm (Sigma)
- SDS 100 gm

Distilled water to 10 litres

Store at room temperature

Composition when diluted 1:10

- Tris 25mM
- glycine 190mM
- 0.1% SDS
- pH 8.3

Method

After polymerisation of the stacking gel, the teflon comb was removed and the wells washed 3 times with distilled water to remove any unpolymerized gel mixture. Fifty µl of the samples were added to the wells, with molecular weight standards added to one or two wells and an equal volume of SDS sample buffer added to any unused wells. The samples were then carefully overlayed with SDS running buffer, an upper tank fitted, and the whole apparatus placed in a gel electrophoresis tank (Biorad) containing SDS running buffer. The upper tank was filled with SDS running buffer containing 200 µl of 0.1% bromophenol blue (Biorad) as tracking dye. The gels were electrophoresed at 30 mA/gel (constant current) until the bromophenol blue tracking line was within 1 cm of the bottom of the gel.

(b) Two-dimensional PAGE

First dimension

Reagents

Anode electrode solution

- 85% orthophosphoric acid 1.35 ml (0.01M) (Ajax Chemicals)
- distilled water 2 litres

Cathode electrode solution

- NaOH 0.8 gm (0.02M)
- 1 litre distilled water

Boil water for 10 minutes, add NaOH in 5 ml of distilled water, boil for additional 5 minutes. Allow to cool.

Method

A cylindrical gel tank (Biorad) was filled with the anode electrode solution. The overlay was "flicked" off the tube gels, they were fitted into the apparatus, and overlayed with a fresh 20 µl of IEF sample buffer and then NaOH cathode solution. The top tank was filled with cathode electrode solution to cover the tubes, and the apparatus connected to the power pack and pre-run for 15 minutes at 200 V, 30 minutes at 300 V, and 30 minutes at 400 V. The tops of the tubes were exposed and the overlay and buffer removed by aspiration. Twenty five µl of the sample was applied, then 10 µl of IEF sample buffer diluted 1:1 with distilled water, followed by NaOH cathode electrode solution. One tube was run without sample for later determination of pH gradient. The

top tank was refilled with cathode electrode solution, and electrophoresis was performed for a total of 4800 volt-hours (constant voltage). The voltage was then turned up to 800 V for 60 minutes, and then turned off.

Second dimension

The tube gels were gently extruded and equilibrated in 5 ml of SDS sample buffer (with or without dithiothreitol) for 2 hours at room temperature with rocking. They were then placed onto a slab polyacrylamide gel which had been prepared as above except that a comb had not been used to produce wells in the stacking gel (apart from one for molecular weight markers). The gel was overlayed with SDS running buffer and electrophoresis proceeded as above. The tube gel without sample was cut into 1 cm pieces which were placed into 1 ml each of distilled water, and the pH of each piece determined to map the pH gradient in the first dimension.

Staining and destaining of gels

Reagents

(1) Staining solution

- Coomassie blue R250 (Biorad) 2 gm
- 50% trichloroacetic acid (Ajax Chemicals) 2000 ml

Stirred overnight and stored in a plastic bottle

(2) Destaining solution

- methanol (Ajax Chemicals) 2000 ml
- glacial acetic acid (BDH Chemicals) 700 ml
- water 7300 ml

Stored at room temperature

Method

The gels were removed from the electrophoresis apparatus, placed into plastic dishes containing the staining solution, and rocked for 20 minutes. The staining solution was then poured off and the gels destained with 2 to 3 changes of destaining solution over several hours or overnight. The gels were placed on filter paper, covered in Glad-wrap, and dried on a Biorad slab gel dryer.

Autoradiography

In a darkroom, the gels were placed against Kodak X-omat AR film inside a Kodak X-omatic film cassette. The films were exposed to the gels for varying lengths of time at -70°C, and developed in a Kodak X-omat developer at the Department of Radiology, The Queen Elizabeth Hospital.

6. "Western blotting" (Burnette 1981)

Electrophoretic transfer

Reagents

(1) Transfer buffer

- Trizma base 15.15 gm (25mM)
- glycine 72 gm (192 mM)
- methanol 1 litre (20%) (optional, added if wishing to transfer low molecular weight proteins)

Distilled water to 5 litres

(2) "Blotto" blocking solution

- non-fat dried skim milk powder 25 gm

PBS to 500 ml

Method

The SDS-PAGE slab gel was prepared as described above except that detergent solubilised membrane extracts from unlabelled cells were electrophoresed without specific immunoprecipitation. The lysate obtained from a minimum of 2 -3 x 10^6 cells was used for each lane. Two lanes were reserved for molecular weight markers, and these were excised from the gel and stained separately with Coomassie blue for later use in determining the molecular weight of the blotted bands. The remaining gel was equilibrated in transfer buffer for 30 minutes, and then placed within a "sandwich" consisting of a Scotch-Brite^R pad (Biorad), 3 thicknesses of filter paper, the gel, a nitrocellulose sheet (Biorad), 3 further thicknesses of filter paper, and another Scotch-Brite^R pad, all of which had been prewetted with transfer buffer. The sandwich was placed in a plastic holder and inserted into a Biorad "Trans-Blot" apparatus with the nitrocellulose membrane facing the anode. The tank was filled with transfer buffer and the transfer performed at 30 V overnight, and for another 1 - 2 hours at 60 V if the buffer contained methanol.

Detection of membrane proteins

Following transfer, the nitrocellulose membrane was placed in "Blotto" blocking solution for 60 minutes to saturate remaining protein binding sites, and then cut into strips corresponding to the SDS-PAGE lanes. The strips were incubated overnight in antibody (neat tissue culture supernatant or ascites diluted 1:500 in Blotto) at room temperature, then washed 3 times for 15 minutes each in excess Blotto. The strips were then incubated for 60 minutes in a 1:200 dilution of biotinylated anti-mouse IgG (Vector Laboratories) at room temperature, followed by 3 further washes in Blotto. The next incubation was in a 1:100 dilution of a 1:1 mixture of avidin DH and biotinylated horseradish peroxidase H (Vector Laboratories) for 60 minutes at room temperature, and another 3 washes in Blotto. Finally, any blotted bands were revealed by pipetting a solution of 0.5 mg/ml diaminobenzidine tetrahydrochloride (Sigma) and 0.01% hydrogen peroxide in 0.05M Tris-HCl buffer (pH = 7.2) onto the strips layed out on a glass plate. A positive reaction was readily detected by a brown stain. The molecular weight of positive bands was estimated using the molecular weight markers excised from the original slab gel. The strips were dried, mounted, and photographed if desired.

7. Stimulation of lymphocytes in culture

Mixed lymphocyte culture (MLC)

A number of 2-way MLCs were performed in this thesis to study the inhibitory effects of monoclonal antibodies. The blood donors were healthy individuals who had previously been HLA-typed, and pairs of donors were chosen that were completely HLA-DR disparate to maximise the lymphocyte proliferation.

Mononuclear cells from the donors were prepared aseptically and resuspended at a concentration of 1 x 10^6 cells/ml in MLC culture medium (RPMI 1640 medium plus 20% heat-inactivated human AB serum). Aliquots of 100 µl of each of the donor cells were mixed together in the wells of a round-bottomed 96 well microtitre plate (Disposable Products, Adelaide, South Australia). Dilutions of monoclonal antibody ascites were also added at this stage (in replicates of 6), and the cells were cultured at 37°C. On day 4 of culture the wells were each pulsed with 1 µCi of tritiated thymidine

(Amersham, UK). After a further 20 hours of culture the cells were harvested with a Skatron cell harvester (Flow Laboratories) and the incorporated counts measured in a Beckman LS 2800 beta counter (Beckman, Palo Alto, California, USA). The results were expressed as an average of the six replicates and as the percentage inhibition with the test antibody compared to an irrelevant control antibody.

Mixed lymphocyte-endothelial reaction (MLER) (Hirschberg et al, 1975)

Cultured HUVEC will induce proliferation of allogeneic lymphocytes in the MLER (see section 2.1), and the inhibitory effects of monoclonal antibodies were examined in this thesis. It was not practical to HLA-type the HUVEC used in these assays, so the degree of HLA disparity between them and the donor mononuclear cells was unknown.

HUVEC were plated onto a gelatin-coated flat-bottomed 96 well microtitre plate at 2 x 10^4 cells/well in 100 µl of medium, and cultured overnight to form a confluent monolayer covering the base of the well. Donor mononuclear cells were isolated as before and resuspended at a concentration of 1 x 10^6 cells/ml in MLER medium (which is identical to the standard HUVEC culture medium but contains heat-inactivated human AB serum instead of FCS). Aliquots of 100 µl of cells were added to the wells containing HUVEC in the same volume of medium, plus the appropriate dilutions of monoclonal antibody ascites (in replicates of 6). The plate was cultured at 37°C and the wells pulsed on day 6 with 1 µCi of tritiated thymidine. After a further 20 hours of culture, the cells were harvested with a Skatron cell harvester and the incorporated counts measured in a Beckman LS 2800 beta counter (Beckman). Control wells containing HUVEC alone and mononuclear cells alone showed that these cells in isolation were minimally proliferative at this time. The results were expressed as an average of the 6 replicates and as the percentage inhibition with the test antibody compared to control antibody.

8. Cell adhesion assays

Cell adhesion to extracellular matrix (ECM) components

The wells of a flat-bottomed 96 well microtitre plate were incubated overnight at room temperature with 10 µg/ml fibronectin (Boehringer Mannheim), 100 µg/ml collagen type I (Sigma), 100 µg/ml collagen type IV

(Sigma), 20 µg/ml laminin (Sigma) or 0.5% gelatin (all diluted in sterile Hanks buffered salt solution). Following washing with PBS, the wells were blocked with 1% bovine serum albumin in PBS for 60 minutes at room temperature, and then washed again with PBS. HUVEC were eluted from their tissue culture flasks with 0.1% EDTA in sterile Hanks, centrifuged twice in plain RPMI 1640, and resuspended in RPMI 1640 at a concentration of 2 x 10^5 cells/ml. Monoclonal antibody was added to cells as dilutions of ascites, and incubated with mixing for 10 minutes at room temperature. One hundred microlitres (ie 2 x 10^4 cells) were added to each well, and incubated at 37°C for 2 hours. Non-adherent cells were washed off by inversion of the plate and "flicking", followed by washing with PBS, and repeating twice. Adherent cells were quantified by counting the number remaining in 5 random high power fields in each well, and expressed as a percentage of the adherent cells in the control wells. All experiments were performed in duplicate.

Cell adhesion to cultured HUVEC

The adherence of various cell types to cultured HUVEC was quantified by labelling the cells with ^{51}Chromium (Cr) (New England Nuclear). In this assay, the added cells and the HUVEC could be separately stimulated, and the effects of monoclonal antibodies directed against either could be measured.

Cultured HUVEC were plated onto gelatin-coated flat-bottomed 96 well microtitre plates as before and cultured overnight to confluence. In some assays, the HUVEC were stimulated with varying concentrations of either IL-1 or LPS for various times (then washed off 3 times with medium) before the adhering cells were added. LPS was used most frequently, but its effects on cultured HUVEC are very similar to those of IL-1 and tumour necrosis factor (TNF) (see discussion in sections 2 and 4). The cells to be studied were isolated and resuspended in RPMI 1640 plus 5% FCS at a concentration of 10 x10^6/ml. They were labelled with ^{51}Cr (10 µCi/10^6 cells) for 60 minutes at 37°C with continual mixing on a rotating plate, followed by washing 3 times in RPMI 1640 plus 1% FCS. The labelled cells were resuspended in the washing medium at a concentration of 2 x 10^6 cells/ml, and 100 µl of the cell suspension (ie 2 x 10^5 cells) was added to the wells containing HUVEC. In addition, 4 aliquots of 100 µl of the labelled cell suspension were placed in Wasserman tubes (Disposable Products) for counting to determine the mean activity of the total number of cells added to each well. In studies where the added cells were stimulated with phorbol 12-myristate 13-acetate (PMA)

(Sigma), this was included in the wells at the time of addition of the cell suspension, and the stimulation took place during the subsequent incubation. Studies of the inhibition of cell adhesion with monoclonal antibodies were performed in two ways. When the antibody bound to an epitope on the adherent cells, it was included (as the appropriate dilution of ascites, typically 1:500) at the same time as the cells were added to the HUVEC-coated wells. Antibodies directed against HUVEC were added to these cells for 60 minutes, before the addition of the adherent cells, then washed off 3 times. The adherent cells and HUVEC were incubated together for 60 minutes at 37°C. The wells were then topped up with RPMI 1640 plus 1% FCS and the top of the plate sealed with acetate plate sealer (Flow Laboratories). It was then centrifuged in an inverted position at 150g for 5 minutes so that the centrifugal force would dislodge the non-adherent cells from the HUVEC monolayer. Keeping the plate in an inverted position, the dislodged cells were "flicked" off, the plate righted, and the wells inspected under an inverted phase contrast microscope (Nikon, Tokyo, Japan) to ensure that the HUVEC monolayer was intact. The labelled adherent cells were lysed with a solution of 1% Triton X-100 in PBS (200 µl/well) for 30 minutes at room temperature, and the lysate then transfered by pipette to Wasserman tubes for counting in a gamma counter (LKB Wallac 1282 Compugamma, Sweden). All experiments were performed in quadruplicate, and the mean counts of adherent cells in each group expressed as a percentage of the mean counts of the total number of cells added to the individual wells.

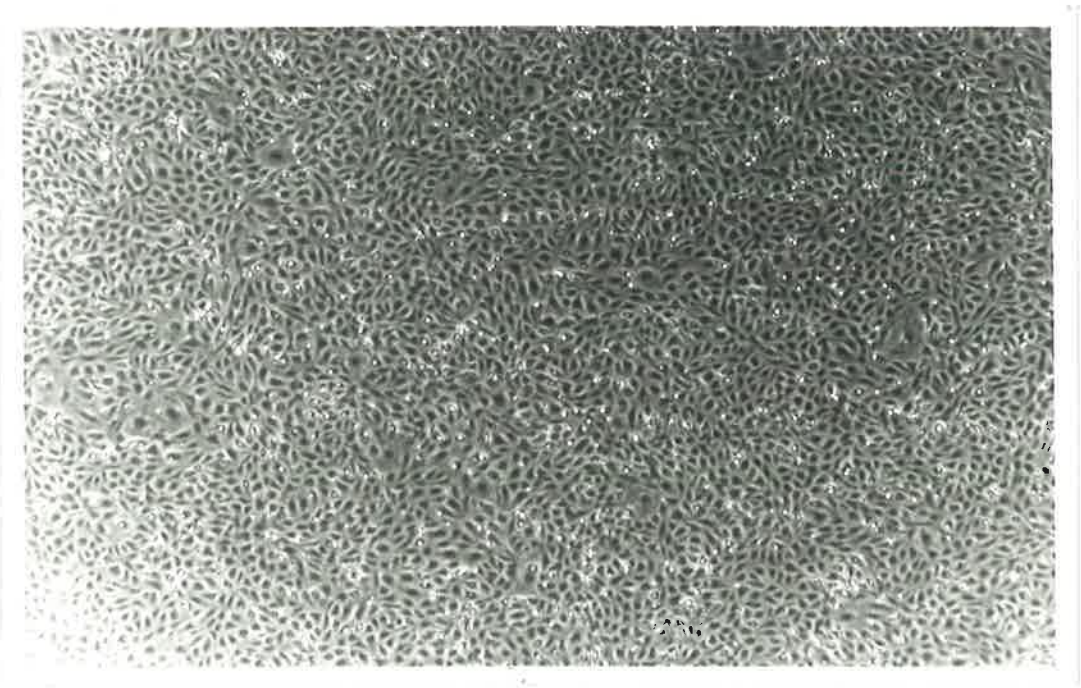
Homotypic cell aggregation

The effects of monoclonal antibodies on the spontaneous and PMA-stimulated aggregation of cells was examined using a simple semi-quantitative assay.

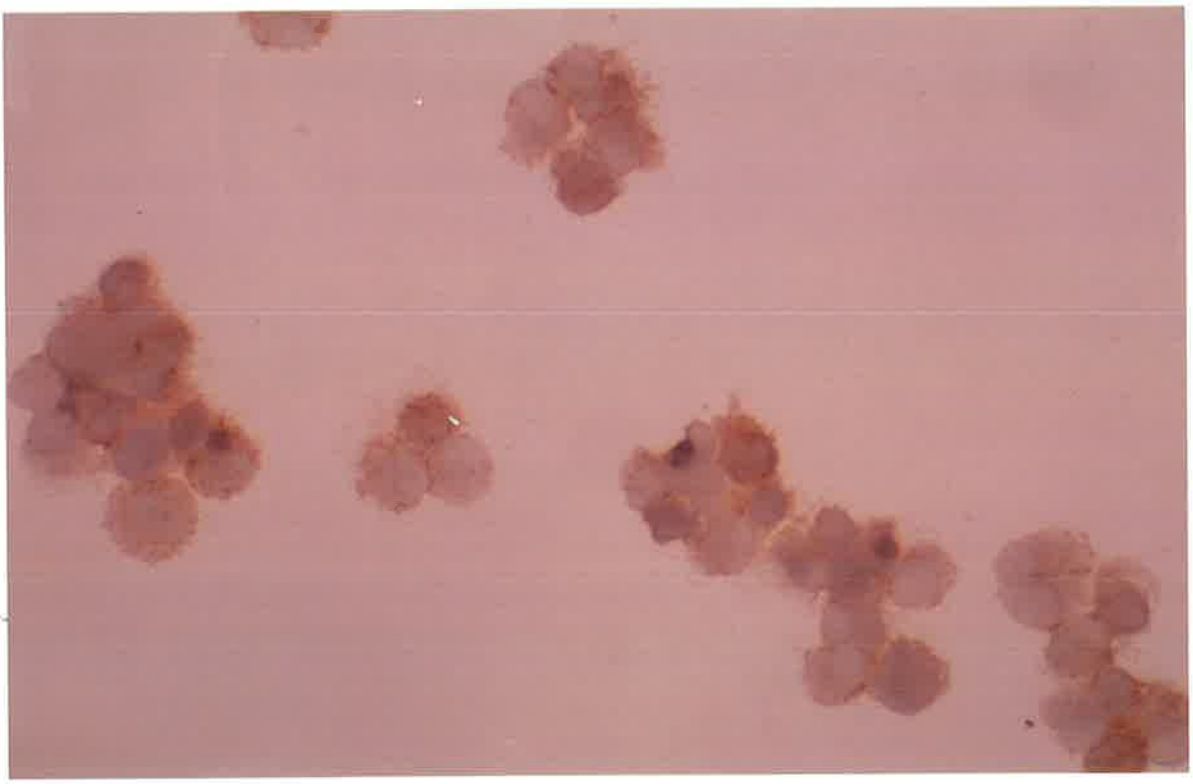
The cells were isolated and resuspended at a concentration of 1 x 10^6 cells/ml in RPMI 1640 plus 10% FCS with pipetting to break up any spontaneously formed aggregates. Aliquots of 100 µl of the cell suspension were placed in the wells of a flat-bottomed 96 well microtitre plate and incubated at 37°C. If PMA or monoclonal antibodies (dilutions of ascites) were to be included in the assay they were added at this time. The wells were observed at various time intervals with an inverted phase microscope and the

degree of aggregation scored semi-quantitatively according to the following scheme:

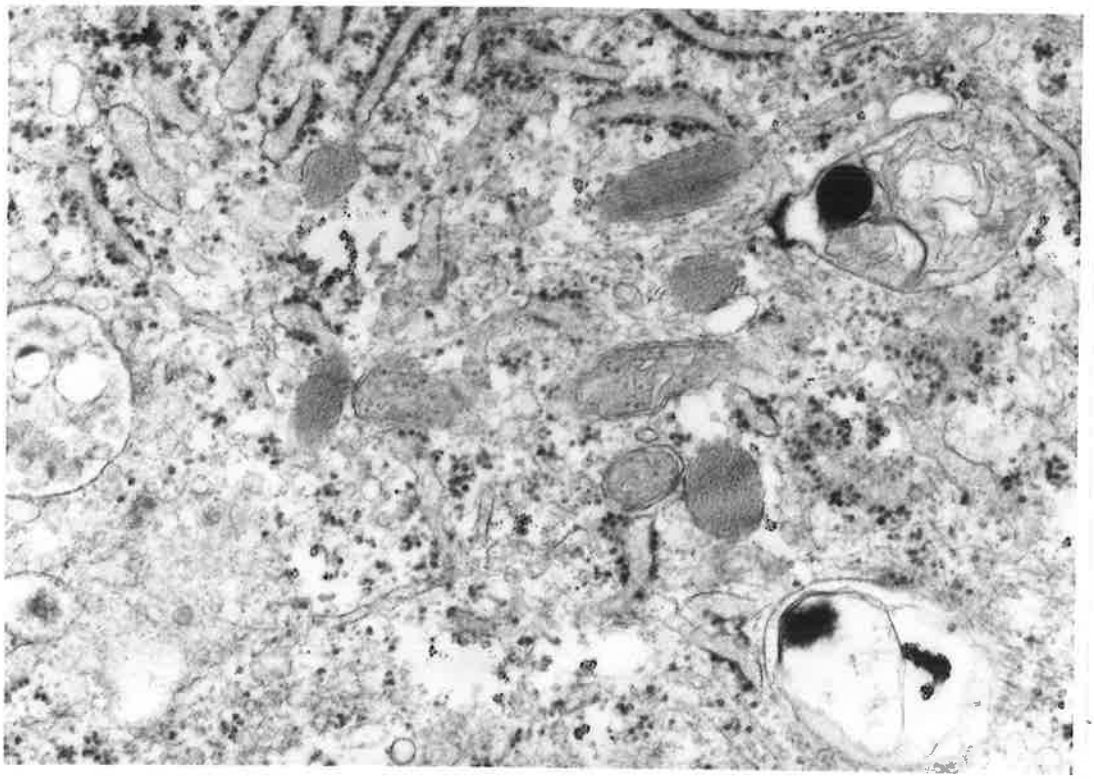
-	no aggregation
+	< 25% of cells aggregated
++	25 - 50% of cells aggregated
+++	> 50% of cells aggregated
++++	all cells aggregated



Cultured HUVEC on the bottom of a tissue culture flask (photographed under an inverted phase contrast microscope, magnification x100)



Isolated cultured HUVEC, positive brown staining for factor VIII antigen (indirect immunoperoxidase, x200)



Weibel-Palade bodies in cultured HUVEC (transmission electron microscopy, magnification x40,000)

SECTION 1:
AN INTRODUCTION TO VASCULAR ENDOTHELIAL CELLS

Introduction

Endothelial cells line all the blood vessels in the body, and they form a continuous organ with a surface area in an average adult of greater than 1000m^2 and weight of more than 100gm (Jaffe, 1987). In most parts of the body they are thin, flattened cells that form a continuous lining separating the blood from the extravascular tissue. However, in the specialised post-capillary venules of lymphoid organs and at some inflammatory sites they have a plumper, cuboidal appearance and bulge into the vessel lumen. These "high endothelial venules" (HEV) will be discussed in more detail in section 4.

The junctions between endothelial cells are usually 10 to 200 nm wide, but can be outside this range in certain tissues (Kaiser and Sparks, 1987). In the brain, testes, and some regions of the thymus the junctions are tight barriers to exchange between the blood and parenchyma. In contrast, the cells lining the glomerular capillaries are separated by gaps of approximately 700 nm that allow the passage of even large molecules. At sites of inflammation the permeability of the endothelial cell lining is increased, which allows the exudation of fluid and proteins and the ready extravasation of leucocytes. The latter contrasts with the infrequent movement of cells from the blood during health. The basement membrane beneath the endothelium forms both a support structure and a secondary barrier to the passage of cells from the blood. This specialised meshwork of extracellular matrix molecules is manufactured by the endothelial cells themselves.

This chapter is a general introduction to endothelial cells and reviews some of their important functions that will not be covered in later chapters. These include their role in coagulation and thrombosis, and an overview of some of the substances synthesised by the endothelium. An effort has been made to concentrate on those that are relevant to the immune response.

A number of techniques exist for studying endothelial cell functions, and those relevant to this thesis will first be briefly reviewed.

Studying the endothelium in vitro

The endothelium of intact blood vessels can be studied by examining sections cut from tissue from vascularised organs. Immunofluorescence or immunoperoxidase techniques utilising specific antibodies will readily identify

the antigens expressed by the endothelium, and can be used to detect changes that occur during pathological states such as inflammation.

Endothelial cells from a number of sources can be maintained in culture, and this is a useful method for studying a number of their functions. However, these cells have a limited life span in culture and usually require special conditions to maintain them in a healthy state. Unfortunately, attempts to develop an immortalised endothelial cell line have had limited success. Human endothelial cells transformed with Simian virus 40 DNA (Gimbrone and Fareed, 1976) or murine sarcoma virus (Faller et al, 1988) have not retained enough of their original characteristics to serve as a generally applicable alternative to non-transformed cells. A hybrid cell line has been created by the fusion of primary human umbilical vein endothelial cells with cells of a human lung carcinoma line (Edgell et al, 1983), and it retains a number of the characteristics of normal endothelium, including the expression of von Willebrand Factor, tissue-type plasminogen activator, and plasminogen activator inhibitor type 1 (Emeis and Edgell, 1988). A murine line obtained by infecting endothelial cells from lymph node stroma with simian virus 40 has also recently been reported (O'Connell and Edidin, 1990). It retains a number of the typical characteristics of endothelial cells, including the expression of factor VIII-related antigen. However, as yet no transformed cell line has received general acceptance for the study of human endothelial cells, and most work continues with non-transformed cells.

Endothelial cells have been successfully isolated from a variety of tissues. Animals are a convenient source, and a significant proportion of endothelial cell research has been performed on serially cultured bovine cells, particularly from the aorta. These cells can be reliably cultured in standard media supplemented with 10% foetal calf serum, and preserve their endothelial phenotypes for many population doublings (Mueller et al, 1980). Cells from large vessels such as this are harvested by enzyme digestion (usually collagenase).

Human microvascular endothelial cells can be isolated from the foreskin (Davison et al, 1980), omentum (Anders et al, 1987), or adipose tissue (Kern et al, 1983). These microvascular cells do not require supplementary growth factors in the culture medium and retain their endothelial phenotype for extended periods, but overgrowth with fibroblasts is generally a problem. They are usually isolated by collagenase digestion of the particular tissue, followed by selective adherence of the endothelial cells to tissue culture vessels.

Human large vessel endothelial cells are most commonly harvested from umbilical veins, but have also been obtained from the aorta, pulmonary artery, or saphenous vein. Umbilical veins are the most convenient source, and again the cells are usually harvested from the lining of the vessel by collagenase digestion (Jaffe et al, 1973a, Gimbrone et al, 1974). These cells are more fastidious than those obtained from other sources, and require a higher proportion of foetal calf serum in the medium plus supplementary growth factors to maintain them for more than just the short term. They are slow growing and eventually lose their characteristic phenotypes and functions with long-term culture. Despite these difficulties, human umbilical vein endothelial cells (HUVEC) remain the most frequent cells used for research, and the information obtained has consistently closely matched that derived from studying the endothelium by other methods.

A specific endothelial cell growth factor (ECGF) has been purified from bovine hypothalamus, after finding that HUVEC grew better if high concentrations of bovine brain or pituitary fibroblast growth factor was added to the medium (Maciag et al, 1979). Specific cell surface receptors for ECGF have been found on murine and human endothelial cells and fibroblasts (Schreiber et al, 1985a). The proteoglycan heparin potentiates its mitogenic activity (Thornton et al, 1983), and binds to it specifically (Maciag et al, 1984). Heparin also restores biological activity to inactivated ECGF, enhances its affinity for its cell surface receptors, and modifies antibody recognition of ECGF, suggesting that it induces a conformational change in the molecule that increases or stabilises its biological activity (Schreiber et al, 1985a). Purification of ECGF using heparin-sepharose affinity chromatography yields a mixture of two single-chain polypeptides with apparent molecular weights of 17kD (α-ECGF) and 20kD (β-ECGF) (Burgess et al, 1985). These belong to a family of polypeptide growth factors, along with acidic fibroblast growth factor and bovine eye-derived growth factor II (Schreiber et al, 1985b). ECGF and heparin are now routinely used in the serial propogation of HUVEC in long-term culture.

Other endothelial cell mitogens have also been described. A platelet-derived endothelial cell growth factor was recently purified and cloned (Ishikawa et al, 1989). The main source is platelets, and it differs from bovine ECGF in that it does not bind to heparin or stimulate fibroblast proliferation. It does stimulate endothelial cell growth as well as chemotaxis in vitro, and stimulates angiogenesis in vivo. The haematopoietic growth factors granulocyte-colony stimulating factor (G-CSF) and granulocyte-monocyte-

colony stimulating factor (GM-CSF) also influence the migration and proliferation of human endothelial cells (Bussolino et al, 1989). Sera from patients with diabetic proliferative retinopathy stimulate human endothelial cell proliferation (Petty et al, 1988). The unidentified causative factor(s) is heat stable and has a molecular weight of less than 15kD.

In contrast, transforming growth factor-β (TGF-β) inhibits endothelial cell regeneration following wounding (Heimark et al, 1986), and inhibits growth factor-induced proliferation and motility in tissue culture (Muller et al, 1987). Endothelial cells express specific TGF-β receptors. It has been postulated that its effect is due to decreasing the level of expression of growth stimulating genes, and therefore altering the cell's response to growth stimulatory factors (Takehara et al, 1987).

These positive and negative influences on endothelial cell growth may have important roles in vivo in situations where angiogenesis occurs, such as wound healing, re-endothelialisation of vessel wall defects, and tumour growth.

HUVEC grow as adherent monolayers of closely apposed, polygonal cells, which gives a characteristic "cobblestone" appearance to the culture. In contrast, fibroblasts and smooth muscle cells grow as overlapping arrays of slender, spindle-shaped cells, which are easily distinguishable from the HUVEC (Jaffe et al, 1973a). Fibroblasts contaminating HUVEC cultures will proliferate faster and eventually take over the culture.

The HUVEC are most commonly grown on gelatin-coated tissue culture plastic, but can be well maintained on fibronectin, collagen, or plastic alone. They can be detached by brief exposure to 0.1%EDTA/0.125%trypsin. The exposure to trypsin must be kept to less than 2-3 minutes otherwise it will digest the cell membrane and decrease viability (Gimbrone et al, 1974). 0.1%EDTA alone for several minutes will also detach the adherent cells. They can be subcultured repeatedly, but eventually their phenotypic characteristics alter and they are steadily less able to form confluent monolayers.

HUVEC can be distinguished from non-endothelial cells by several criteria apart from their characteristic appearance. Endothelial cells synthesise and express factor VIII antigen and von Willebrand factor (vWF) in vivo and in vitro (Jaffe et al, 1973b, Jaffe et al, 1974), and these markers can be localised by immunofluorescence or immunoperoxidase techniques using specific antibodies. Another specific marker of endothelium is the Weibel-Palade body (Weibel and Palade, 1964), which is a rod-shaped tubulated structure found in the cytoplasm by electron microscopy. These organelles are the intracellular

storage site for vWF (Wagner et al, 1982) and granule membrane protein-140 (GMP-140) (McEver et al, 1989), which is also found in platelets (see section 4). Both of these proteins rapidly translocate to the cell surface after activation of the endothelial cells (Hattori et al, 1989). Cultured endothelial cells express ABO blood group antigens, unlike fibroblasts or smooth muscle cells (Jaffe et al, 1973a). Another histological marker for endothelial cells is the Ulex europaeus I agglutinin, a lectin specific for some α-L-fucose-containing glycocompounds (Holthofer et al, 1982). This specific binding has been utilised by some investigators to purify endothelial cells from a mixed culture of cells.

The study of endothelial cells using these techniques has yielded considerable information about the role of the endothelium in homeostatic processes. They are metabolically active cells which possess the necessary organelles for the processing and synthesis of proteins and a number of bioactive substances. The majority of this thesis concerns the cell surface molecules involved in endothelial interactions with leucocytes and the extracellular matrix, and how they participate in the immune response to a vascularised allograft. The endothelium is also intimately involved in the important processes of coagulation and thrombosis, and this function will be briefly reviewed because it is of some relevance to allograft rejection.

The endothelium in coagulation and thrombosis

The endothelium actively contributes to the control of clotting, platelet activation, and clot dissolution. It normally presents a non-thrombogenic surface, and is non-adherent for unstimulated platelets.

There exist two distinct endothelial mechanisms for preventing activation of the coagulation system. The first is the synthesis of antithrombin III (Chan and Chan, 1979), which binds to heparan sulphate on the cell surface (Stern et al, 1985a). This dramatically enhances the ability of antithrombin III to inactivate thrombin, by forming a covalent thrombin-antithrombin complex which is then rapidly cleared by the liver (Lollar and Owen, 1980). The second mechanism is the synthesis of thrombomodulin, which binds to thrombin and so decreases its ability to catalyse clot formation on the cell surface (Esmon, 1987). This event also markedly enhances the thrombin-induced activation of protein C, which acts as an anticoagulant by inactivating factors Va and VIIIa, two of the regulatory proteins of the coagulation pathway. These two proteins are required for the normal function of two coagulation proteases, IXa and Xa

(Esmon, 1987), both of which can also bind to endothelial cells (Stern et al, 1983).

The endothelium can also influence coagulation by actively modifying its interactions with platelets. The synthesis of prostacyclin (PGI_2) by endothelial cells (Weksler et al, 1977) is stimulated by numerous substances and events, including thrombin (Weksler et al, 1978), leukotriene C (Cramer et al, 1983), and immunological injury (Goldsmith et al, 1984). Prostacyclin inhibits the adhesion of stimulated platelets to the endothelium (Fry et al, 1980). Its synthesis is inhibited by aspirin (Jaffe and Weksler, 1979) and other non-steroidal antiinflammatory drugs such as indomethacin (Weksler et al, 1977). Aggregating platelets release adenosine diphosphate (ADP), which recruits other platelets into the platelet plug, and adenosine triphosphate (ATP), which is a vasodilator. Their effects are modulated by rapid metabolism by ectoenzymes synthesised by the endothelium (Pearson et al, 1980).

The endothelium participitates in fibrinolysis by synthesising the two forms of plasminogen activator, urokinase-type and tissue-type (which is active only after binding to fibrin) (Loskutoff and Mussoni, 1983), as well as their receptors (Miles et al, 1988). The two plasminogen activators are protected from their inhibitors when bound to these receptors. They cleave plasminogen to form plasmin, the principal fibrinolytic enzyme. Tissue type plasminogen activator release is stimulated by thrombin (Levin et al, 1984). Endothelial cells also secrete plasminogen activator inhibitor, which is partly neutralised by protein C (Esmon, 1987), enhancing fibrinolysis. Lipoprotein (a) interferes with endothelial cell fibrinolysis by inhibiting plasminogen binding and hence the generation of plasmin. It is also known to accumulate in atherosclerotic lesions (Hajjar et al, 1988), which may provide a link between impaired cell surface fibrinolysis and progressive atherosclerosis.

In some pathological states vascular thrombosis is a prominent feature, and there is evidence that in such situations the endothelium can be shifted towards a procoagulant state. Cultured endothelial cells stimulated by IL-1, TNF, or lipopolysaccharide (LPS), produce tissue factor (thromboplastin)-like procoagulant activity, which accelerates the ability of factor VII to activate factor X (Bevilacqua et al, 1984, Bevilacqua et al, 1986a, Nawroth and Stern, 1986, Stern et al, 1985b, Colucci et al, 1983). At the same time the endothelial cell dependent activation of protein C is depressed, reducing its anticoagulant effect. These procoagulant effects are potentiated by cyclosporine, which has been implicated in thrombotic episodes in transplanted organs (Carlsen et al, 1988). IL-1, TNF, and LPS also decrease fibrinolysis by inhibiting tissue type

plasminogen activator and stimulating plasminogen activator inhibitor (Bevilacqua et al, 1986b, Nachman et al, 1986, van Hinsbergh et al, 1988). Therefore, the net effect of these cytokines is to encourage coagulation, which contributes to the thrombotic state that exists at sites of inflammation.

Another procoagulant effect of the endothelium is the synthesis of factor V, which accelerates the activation of prothrombin by factor Xa (Cerveny TJ et al, 1984). Factors IXa and X also bind to the endothelium, which helps to localise the coagulation process rather than it spreading along the vessel (Stern et al, 1983, Heimark and Schwartz, 1983).

Exposure of the subendothelium after damage to the endothelium is rapidly followed by the adhesion of stimulated platelets. They either bind directly to the subendothelial collagen via specific collagen receptors (Coller et al, 1989), or more importantly, bind to vWF which in turn anchors to the subendothelial matrix (Meyer et al, 1983). Platelets possess a specific vWF receptor (Kao et al, 1979), and the vWF in turn binds to collagen types I, III, IV, and V in the matrix (Meyer et al, 1983). Significantly, platelet adhesion to the subendothelium is decreased in von Willebrand disease, which is characterised by reduced activity of von Willebrand factor. Active vWF is synthesised by the endothelium (Jaffe et al, 1974), and its secretion is induced by thrombin and complement proteins C5b-9 (Levine et al, 1982, Hattori et al, 1989).

It is clear that the endothelium has a central role in the processes of coagulation and thrombosis. It normally serves to discourage clotting, but injury and the effects of inflammation convert it to a more thrombogenic surface. Therefore it is not surprising that the vigorous inflammation which accompanies allograft rejection is often complicated by vascular thrombosis, and the consequent ischaemia may ultimately be the prime reason for the loss of many grafts.

A selection of relevant endothelial synthetic functions

The endothelium possesses considerable synthetic ability, and a number of these processes that are relevant to this thesis will be briefly covered.

The endothelium sits on a supporting matrix formed by extracellular matrix proteins. Many of these are produced by the endothelial cells themselves, including collagen of various types (Sage et al, 1981), fibronectin (Jaffe and Mosher, 1978), thrombospondin (Mosher et al, 1982), elastin (Mecham et al, 1983), and laminin (Gospodarowicz et al, 1981). The relationship between the

endothelium and the extracellular matrix will be discussed in more detail in section 3.

Cultured endothelial cells stimulated with the phorbol ester 12-o-tetradecanoyl phorbol-13-acetate (TPA) synthesise collagenase (Gross et al, 1982). This production of this enzyme, which has the potential to modify the adjacent matrix (including the basement membrane), may be significant in the vascular remodelling that occurs during wound healing and neovascularisation.

Several vasoactive substances are produced by the endothelium. Angiotensin-converting enzyme, which hydrolyses angiotensin 1 to the vasoconstrictor angiotensin 2 and inactivates the vasodilator bradykinin, is synthesised by the endothelium in the lung (Das and Soffer, 1975). The endothelium also contains angiotensinases which ultimately inactivate the angiotensin 2 (Kumamoto et al, 1981). Vasoactive agents such as acetylcholine and bradykinin stimulate the endothelium to release a short-lived endothelium-derived relaxing factor (EDRF), which is probably nitrous oxide, resulting in vasodilatation (Palmer et al, 1987). A potent endothelium-derived vasoconstrictor peptide known as endothelin has also been isolated (Yanagisawa et al, 1988).

Cultured endothelial cells secrete growth-promoting activity, much of which is attributed to platelet-derived growth factor (PDGF), a 30kD glycoprotein that is a vasoconstrictor and both chemotactic and mitogenic for vascular smooth muscle cells (DiCorleto and Pope, 1983). This secretion is stimulated by TNF (Hajjar et al, 1987), TGF-β (Daniel et al, 1987), thrombin (Harlan et al, 1986), activated factor X (Gajdusek et al, 1986), and injury (Fox and DiCorleto, 1984). A commercial fish oil extract (max EPA [Solgar]) almost completely inhibits the production of PDGF (Fox and DiCorleto, 1988).

Injury also stimulates endothelial cells to express both Fc and C3b receptors (Ryan et al, 1981), as does infection with herpes simplex virus (Cines et al, 1982). They are not expressed normally by healthy endothelial cells in culture.

Stimulating cultured endothelial cells with cytokines and other bioactive substances induces a number of changes that are relevant to inflammation. Leukotrienes C4 and D4 (McIntyre et al, 1986), thrombin (Prescott et al, 1984), IL-1 (Bussolino et al, 1986), and TNF (Camussi et al, 1987) all induce production of platelet-activating factor (PAF), a highly potent lipid mediator of inflammation and cell-cell interactions. TNF, LPS, thrombin, and IL-1 stimulate cultured endothelial cells to synthesise endogenous IL-1 (Nawroth and Stern,

1986, Kurt-Jones et al, 1987, Miossec et al, 1986a, Libby et al, 1986, Warner et al, 1987, Stern et al, 1985b). This is potentiated by γ-interferon (Miossec et al, 1986).

Supernatant taken from cultured human endothelial cells has strong growth promoting activity for hybridoma cells (Astaldi et al, 1980), and this is due to production of IL-6, which is increased by stimulation with IL-1 (Sironi et al, 1989), TNF, lymphotoxin (LT), or LPS (Jirik et al, 1989). γ-IF enhances the effects of these factors on IL-6 production (Leeuwenberg et al, 1990). TNF, LPS, and IL-1 also stimulate cultured HUVEC to produce IL-8, best known as a chemotactic factor for neutrophils (Strieter et al, 1989). Human vascular cells can stimulate granulopoiesis in agar culture of human bone marrow cells (Knudtzon and Mortensen, 1975), and they produce colony-stimulating activity, which is increased by LPS (Quesenberry and Gimbrone, 1980). IL-1 and TNF induce the production of GM-CSF, G-CSF, and M-CSF (Broudy et al, 1986, Bagby et al, 1986, Segal et al, 1987, Broudy et al, 1987a, Broudy et al, 1987b, Zsebo et al, 1988, Fibbe et al, 1989). The human colony-stimulating factors have multiple biological effects, including direct enhancement of the activities of neutrophils, eosinophils, and macrophages (Clark and Kamen, 1987). Those produced by endothelial cells after stimulation by IL-1 and TNF may locally modulate the function of these effector cells of the immune system, as well as stimulate the growth of haematopoietic precursors. Endothelial cells are induced to produce GM-CSF, G-CSF, and M-CSF by modified low-density lipoproteins (Rajavashisth et al, 1990), which also stimulate monocyte adherence to the endothelium. This might be relevant to the pathogenesis of atherosclerosis.

The endothelium may also contribute directly to tissue injury at the site of inflammation. Both IL-1 and γ-interferon induce the production of superoxide anion (O_2^-) by cultured endothelial cells (Matsubara and Ziff, 1986), which might add to the damage caused by its production by infitrating phagocytes (reviewed by Henson et al, 1987).

The endothelium is clearly a metabolically active and productive organ that is a central participant in coagulation and thrombosis. The principal aim of this thesis is to examine its role in the immune response, particularly that generated against a vascularised allograft. The interactions of the endothelium with the cells and supporting matrix in its adjacent microenvironment are controlled by a complex collection of receptors expressed on the cell surface. The part played by the endothelium in

generating an alloimmune response and its expression of MHC molecules will be examined in section 2. The next section deals with endothelial receptors that bind to components of the extracellular matrix, and section 4 covers the receptors that mediate the interactions between the endothelium and circulating leucocytes.

SECTION 2:
THE ENDOTHELIUM AS A STIMULATOR AND A TARGET OF THE ALLOIMMUNE RESPONSE

2.1 Literature Review

Introduction

The stimulus for an alloimmune response is the recognition by the recipient's immune system of foreign antigens on the transplanted tissue. In a vascularised allograft, the endothelium presents a large surface area of foreign cells readily accessible to the host immune system. As a consequence, the endothelium is regularly a major site of damage during allograft rejection. It has been postulated that necrosis and transplant loss due to rejection is primarily due to severe microvascular injury and subsequent ischaemia (Dvorak et al, 1986).

The chief determinants of the recognition of foreign tissue (ie non-self) are a polymorphic family of cell surface glycoproteins known as the major histocompatibility antigens, which are encoded by a cluster of genes called the Major Histocompatibility Complex (MHC). In humans, the molecules encoded by the MHC are usually referred to as the human leucocyte antigens (HLA), and the genes are grouped together on the short arm of chromosome 6. The immunogenicity of an allograft is largely determined by the degree of incompatibility between the donor and recipient HLA antigens.

This chapter reviews the factors that determine the immunogenicity of the endothelium, and discusses the evidence that endothelial cells are fully competent antigen-presenting cells.

Polymorphic antigen systems on human endothelium

The HLA and ABO blood group systems, which are the two most important histocompatibility systems in humans, are both expressed on the vascular endothelium. A less well characterised group of polymorphic antigens found on the endothelium is known as the endothelial-monocyte (EM) system. It is important in a minority of cases of allograft rejection.

(1) HLA system

The loci for the HLA system are divided into two groups which encode structurally distinct sets of antigens. The class I antigens, coded for by the A, B, and C loci, were the first described (Kissmeyer-Nielsen et al, 1968). They are cell surface glycoproteins consisting of a polymorphic heavy chain non-covalently bound to a constant light chain. The heavy chain varies from 338 to

341 amino acids and has a molecular weight of 45 kD. The light chain, known as β-2 microglobulin, has a 99 amino acid backbone and a molecular weight of 12 kD. Virtually all nucleated cells express HLA class I antigens.

The HLA class II antigens are also cell surface glycoproteins. They consist of a heavy (α) chain non-covalently bound to a light (β) chain. The α chain has a 229 to 232 amino acid backbone and a molecular weight of 35 kD. The β chain is 229 to 237 amino acids long and weighs 28 kD. Most of the polymorphism resides in the β chain. The cell distribution of the class II antigens is less widespread than that of class I antigens, and they are normally confined to B lymphocytes, activated T lymphocytes, monocytes/ macrophages, dendritic cells, and the vascular endothelium. The class II region in the MHC is more complex than the class I region, and is divided into genes encoding DR, DQ, and DP antigens, which are expressed on the cell surface, and a number of pseudogenes which do not produce detectable protein (Trowsdale, 1988).

Each of the HLA loci exhibit considerable polymorphism, generating an enormous potential number of combinations. Therefore, randomly selected individuals are likely to have little or no similarity between their HLA types. However, as the genes are inherited in a Mendelian fashion with very little crossover, family members frequently share haplotypes and there is approximately a one in four chance that siblings will be HLA-identical. The polymorphism is generated by differences in the amino acid sequences of the individual chains.

The mechanism by which HLA incompatibility leads to allorecognition can best be understood by examining the role of the HLA antigens in the body's immune response to foreign antigen eg in microbial or viral infection.

The surveillance cells of the immune system are the T lymphocytes. They possess a unique receptor (the T cell receptor - TCR) which specifically recognises antigen that has been processed by specialised antigen-presenting cells (APC). By mechanisms that are still incompletely understood, T lymphocytes that recognise self antigens (and so are potentially autoreactive) are deleted and/or inactivated in the thymus. The remaining T lymphocytes have TCR which can recognise non-self antigens. Many cells fail to survive thymic selection, but an extremely large repertoire still remains which potentially is able to recognise any foreign antigen.

A crucial finding which helped elucidate this process was that T lymphocyte recognition required both the foreign antigen and the body's own MHC molecules (MHC restriction) (Zinkernagel and Doherty, 1974). APC

process the antigen to a peptide fragment 10 to 20 amino acids long which is bound to the MHC molecule (Gotch et al, 1987), and only in that form is it recognisable by the TCR. Fragments of intracellular proteins (eg viral, tumour) are usually presented associated with class I molecules (endogenous pathway), and fragments from soluble extracellular proteins are associated with class II. Recent evidence suggests that class II molecules in some circumstances are also able to bind fragments from endogenous proteins (Nuchtern et al, 1990) and that exogenous antigen can be presented with class I molecules (Rock et al, 1990). The complexes formed by class I molecules and antigen fragments are the prime targets for the CD8-positive cytotoxic T lymphocytes, and the class II molecule complexes are targets for the CD4-positive T lymphocytes (Bach et al, 1976).

The structure of the HLA-A2 antigen has been elucidated by x-ray crystallography (Bjorkman et al, 1987a). The sites of most of the polymorphic amino acids are clustered on top of the molecule in a large groove formed by the $\alpha 1$ and $\alpha 2$ regions of the heavy chain (Bjorkman et al, 1987b). The floor of the groove is formed by 8 strands of β-pleated sheets, and it is walled by two α-helical segments. The groove is large enough to accommodate a peptide of 10 to 20 amino acids, depending on its configuration, and appears to be the binding site for processed peptide. Unidentified electron-dense material within the groove which co-crystallised with the HLA-A2 molecule may represent bound peptide. A number of polymorphic residues exist in the floor of the groove, and these are well placed to interact with the peptide fragment. Other polymorphic residues that are placed on the outer edge of the groove face outwards and can interact with the TCR. It is hypothesised that the TCR recognises and binds to a complex formed by the combination of the histocompatibility protein and the processed antigen.

A hypothetical structure of the HLA class II molecule based on the HLA-A2 structure has been calculated (Brown et al, 1988). This model is very similar to the structure of HLA-A2, and has a single binding site formed by the highly polymorphic amino-terminal domains of the $\alpha 1$ and $\beta 1$ chains. The most polymorphic residues are also clustered around the groove that is formed. There is no published structure for class II molecules as yet, as it has been difficult to produce crystals of sufficient quality for analysis.

The enormous polymorphism of the HLA system provides a mechanism for increasing the number of peptides that can be presented to the host's T lymphocytes, and so covering the large number of potential foreign antigens in the environment. The processed peptides bind to different HLA molecules with

varying affinities and create different conformations, which offers an explanation for the variation between individuals of susceptibility to pathogenic microorganisms and for the known HLA associations with different diseases.

The TCR which binds to the peptide/MHC complex is composed of disulphide-linked α and β polypeptides, each about 40 to 50 kD in size (Marrack and Kappler, 1987). A second type of TCR that has different chains (γ and δ) is expressed by about 1 to 10% of peripheral T lymphocytes and by the majority of T cells in the skin. Its function is uncertain (Davis and Bjorkman, 1988). The α and β chains of the classical TCR consist of constant and variable regions. The highly polymorphic variable regions can give rise to as many as 10^{15} combinations to cover the tremendous diversity of antigen recognition that is required. The variable regions interact with the peptide fragment in the binding groove and with adjacent residues on the HLA molecule.

The TCR is associated on the cell surface with a collection of invariant proteins known as the CD3 complex. This is thought to have a role in transmitting information from the TCR to the inside of the cell (Marrack and Kappler, 1987). An intact TCR/CD3 complex is necessary for the expression of either component on the cell surface.

The TCR/HLA/peptide fragment interaction and the MHC restriction presumably evolved to allow the recognition of foreign peptides in association with the body's HLA molecules in order to destroy infected cells (Krensky et al, 1990). Recognition and rejection of an allograft apparently occurs outside of the rules of MHC restriction. Foreign HLA is recognised in association with bound peptide by recipient T lymphocytes (Margulies, 1989), although the peptides may be of donor or recipient origin. The particularly novel conformation provided by the peptide bound to different HLA probably accounts for the large percentage of T lymphocytes that are stimulated by foreign HLA (Matzinger and Bevan, 1977), and for the large fraction (at least 10%) of clones of T lymphocytes derived from biopsies of rejecting grafts that are donor reactive (Bonneville et al, 1988, Kurnick et al,1987). This contrasts with the small number of T lymphocytes that respond to foreign peptide bound to self HLA (Townshend and McMichael, 1987). However, it is still possible that the presentation of foreign antigens as peptides in association with self MHC on recipient cells makes some contribution to the development of alloreactivity. These recipient cells can either be those infiltrating the graft or be at distant sites (eg draining lymph nodes).

A number of non-antigen specific accessory molecules contribute to the interaction between the TCR and the HLA/antigen complex. These will be discussed in more detail in sections 3 and 4.

(2) ABO blood group antigens

Vascular endothelium in vivo (Paul et al, 1981) and endothelial cells in culture (Jaffe et al, 1973a) express ABO blood antigens, and it is generally accepted that it is unwise to cross this barrier in transplantation. The early experience with ABO-incompatible grafts was frequent, early, and severe rejection directed against the vascular endothelium, as most individuals possess antibodies against the blood group that they lack. Other blood group antigens are generally not thought to be significant, although there has been some suggestion that Lewis antigens constitute a histocompatibility system in clinical renal transplantation (Paul et al, 1981).

(3) EM system antigens

The EM system is a distinct polymorphic antigen system that is confined to endothelium and monocytes (Moraes and Stastny, 1977). Sera from some kidney transplant recipients was found to contain antibodies reactive with endothelial cells and monocytes but not lymphocytes which were distinct from anti-HLA antibodies. These anti-EM antibodies recognise antigens encoded by loci that segregate with HLA (Neppert et al, 1985). Other sera have been found that contain antibodies reactive with granulocytes, monocytes, and endothelial cells (Thompson et al, 1980), but it is unclear whether they are of any clinical significance.

A number of studies have shown that these antigens have some relevance to clinical transplantation. Paul et al (1979a, 1979b) found anti-endothelial antibodies in the sera of a significant number of patients with rejection of kidney allografts. The sera bound in indirect immunofluorescence and indirect immunoperoxidase techniques specifically to the peritubular capillaries in biopsies taken from the grafts after transplantation (Paul et al, 1979d). They also described a patient with pretransplantation anti-EM antibodies who underwent accelerated rejection of a renal allograft (Paul et al, 1979c). Absorption experiments confirmed that these antibodies were not directed against HLA antigens. Another group found a significant incidence of anti-EM antibodies in patients who rejected renal allografts (Cerilli et al, 1981, 1985a, 1988) and cardiac allografts (Brasile et al, 1985). They screened for antibodies against a panel of monocytes and endothelial cells from human

umbilical veins. The incidence of these antibodies is low (less than 10%) and they usually occur in association with anti-HLA antibodies. It has been suggested that they can mediate rejection that occurs in transplants taken from HLA identical siblings. The same group has also identified these antibodies in patients with atherosclerosis and systemic vasculitis (Cerilli et al, 1985b, Cerilli et al, 1987, Brasile et al, 1989). Monoclonal antibodies that react exclusively with antigens on endothelial cells and monocytes have been described (Wood et al, 1988, Schook et al, 1987). The relationship of these antigens to EM antigens is unknown.

Patients have been described who developed severe vascular rejection mediated by anti-endothelial cell antibodies that did not react with monocytes (Jordan et al, 1988, Miltenburg et al, 1989). An endothelial-specific antigen system relevant to allograft rejection has also been defined in the rat (Paul et al, 1983).

The EM antigens appear to be of limited importance in clinical transplantation. Rejection occurring after HLA-identical transplantation may be due to anti-EM antibodies, but this is an uncommon situation. In most instances, the antibody response to HLA incompatibility is significantly greater than the response to EM incompatibility. The standard microcytotoxicity and cross-matching techniques used for matching a renal allograft use lymphocytes as the target cells, and will not detect antibodies that only bind to endothelial cells or monocytes. Patients who have developed a high titre of anti-HLA antibodies due to previous exposure from a transplant, transfusion, or pregnancy (highly sensitised) may also possess anti-EM antibodies. It has been suggested that such patients should also be matched by non-standard techniques such as monocyte or endothelial cell crossmatching to detect any anti-EM antibodies (Cerilli et al, 1985a). An alternative suggestion is the use of indirect immunoperoxidase staining of sections from the donor kidney with the recipient's serum to detect antibodies that bind to the vascular endothelium (Evans et al, 1985c).

The role of the endothelium in antigen presentation

Improvements in crossmatching techniques and the avoidance of ABO blood group incompatibilities have dramatically reduced the incidence of rejection of vascularised allografts due to antibodies that were present before the transplant took place. Most episodes of rejection now are initiated by T

lymphocyte responses, although antibody-mediated damage can still have a secondary contribution.

The fundamental event that initiates such an immune response is the activation of CD4-positive helper T lymphocytes. This is a result of the interaction between the TCR and HLA class II antigens on the surface of specialised antigen-presenting cells (APC) (Unanue and Allen, 1987). Following activation, the T lymphocytes produce a number of cytokines which amplify the immune response. Their effects include B lymphocyte activation, upregulation of HLA class II molecules on APC, and the activation of cytotoxic T lymphocytes, which are then able to recognise their target antigens presented in association with HLA class I molecules. Therefore, a primary immune response is usually limited to antigens presented in association with HLA class II molecules.

An alloimmune response in the presence of a disparity of HLA class II arises from direct activation of CD4-positive helper T lymphocytes. Disparity of HLA class I alone (ie with HLA class II identity) also generates a response, albeit usually much weaker than when there is a combined disparity (Bach et al, 1976). Several observations have provided possible explanations for this phenomenon. HLA class I antigens themselves may be able to activate helper T lymphocytes (Reinsmoen et al, 1983). The recipient's own APC may be able to present fragments of the donor class I antigens in association with class II to helper T lymphocytes (Golding and Singer, 1984). Some CD8-positive cytotoxic T lymphocytes appear to be helper cell independent, and produce their own lymphokines (Wee et al, 1982). Conversely, some CD4-positive T cell clones also have cytotoxic activity (Spits et al, 1982). Consequently, either CD4-positive or CD8-positive T lymphocytes alone are able to transfer rejection of murine skin grafts, provided that the graft and recipient differ in class II or class I antigens, respectively (Colvin, 1990).

In non-vascularised allografts such as skin grafts the predominant APC appear to be the highly specialised dendritic cells which are normally resident in the skin. There is divergance of opinion about the major APC in vascularised allografts. One theory invokes the "passenger leucocytes", subsequently identified as dendritic cells (Lafferty et al, 1983, Austyn, 1987, Austyn and Larsen, 1990). In several animal models of allograft rejection, removal of the passenger leucocytes by various manipulations significantly prolonged the graft survival (Hart et al, 1980, Lafferty et al, 1975). However, this improvement is very dependent on the strain combination used and is most successful when other tissues in the graft (eg vascular endothelium) do

not constitutively express class II antigens (Hart and Fabre, 1981a, Mason and Morris, 1986). Depletion of passenger leucocytes is a better form of immunosuppression in non-vascularised grafts (eg isolated pancreatic islets) than in vascularised grafts (Stegall et al, 1990).

Macrophages and B lymphocytes also are APC (Unanue and Allen, 1987), although resting B lymphocytes are unable to activate resting T lymphocytes (Lassila et al, 1988). The probable chain of events is that a non-B lymphocyte APC is required to initially activate the helper T lymphocyte, which in turn activates the antigen-specific B lymphocytes which bear the same antigen-MHC combination as that on the original APC (DeFranco, 1988). Studies with murine B cell lines also support the notion that B lymphocytes are "incomplete" APC as exogenous IL-1 was required to produce an alloreactive response (Glimcher et al, 1982).

The endothelium in human vascularised allografts constitutively expresses HLA class II antigens, and a number of studies show that endothelial cells in culture are fully functional APC. Cultured endothelial cells are able to present protein antigens to sensitised T lymphocytes (Hirschberg et al, 1980, Hirschberg et al, 1982, Nunez et al, 1983, Wagner et al, 1984, Wagner et al, 1985a, McCarron et al, 1986), and can augment T lymphocyte responses to mitogens, which is dependent on the participitation of a non-responding accessory cell (Ashida et al, 1981, Shanahan et al, 1985, Guinan et al, 1989). The experiments of Guinan et al showed that the addition of endothelial cells dramatically augmented the production of IL-2 by the T lymphocytes. Direct contact between the cells was required (Hughes et al, 1990).

Most significantly, cultured endothelial cells are able to evoke a primary allogeneic response (Hirschberg et al, 1975, Burger et al, 1982, Pober et al, 1983b, Groenewegen et al, 1984a, Groenewegen and Buurman, 1984b, Geppert and Lipsky, 1985). This is an important property of endothelial cells, as other cell types such as fibroblasts and smooth muscle cells can be induced to express HLA class II antigens (Pober et al, 1983b, Geppert and Lipsky, 1985, Pober et al, 1986a) and present nominal antigen and alloantigens to sensitised T lymphocytes (Geppert and Lipsky, 1985, Pober et al, 1986a) or T cell clones (Umetsu et al, 1986), but are unable to support a primary immune response (Pober et al, 1983b, Geppert and Lipsky, 1985, Pober et al, 1986a, Umetsu et al, 1986). In addition, they cannot substitute for endothelial cells or other accessory cells in supporting mitogen-induced lymphocyte proliferation (Ashida et al, 1981, Shanahan et al, 1985) or

augmenting IL-2 production (Guinan et al, 1989). Fibroblasts and other cell types can be induced to express similar levels of HLA class II antigens as endothelial cells, which indicates that the binding of the TCR to the MHC/processed antigen complex alone is insufficient to activate resting helper T lymphocytes. Geppert and Lipsky (1987) showed that fibroblasts expressing HLA class II (after stimulation with γ-interferon) are able to take up and process antigen effectively. Their failure to present antigen to resting T lymphocytes is reversed by the addition of exogenous IL-2, showing that these fibroblasts still are unable to generate the signal that stimulates production of IL-2 by resting T lymphocytes (Umetsu et al, 1986). Cultured endothelial cells behave as fully competent APC, and therefore must be capable of providing the co-stimulatory signals these other cells cannot.

The identity of the additional signal (or signals) is uncertain. In certain models it appears to be IL-1 (Unanue and Allen, 1987), but exogenous IL-1 (or the transfer of conditioned medium) cannot induce HLA class II-expressing fibroblasts to stimulate a primary allogeneic response (Pober et al, 1983b, Umetsu et al, 1986), and dendritic cells (which are fully competent APC) do not produce significant amounts of IL-1 (Koide and Steinman, 1987). Endothelial cells produce IL-1 after stimulation with LPS (Miossec et al, 1986a, Libby et al, 1986, Wagner et al, 1985b), TNF (Libby et al, 1986, Locksley et al, 1987, Nawroth and Stern, 1986), and IL-1 itself (Warner et al, 1987). The production of IL-1 is enhanced by the addition of γ-interferon (γ-IF) (Miossec et al, 1986b). Endothelial cells may differ from fibroblasts in that they express a membrane-associated form of IL-1 after TNF or LPS stimulation (Kurt-Jones et al, 1987, Unanue and Allen, 1987), which might provide a specific signal that cannot be reproduced by soluble IL-1.

Another soluble factor produced by endothelial cells that may have co-stimulatory function during antigen presentation is IL-6. It synergises with IL-1 in the stimulation of mitogen-induced and anti-CD3-induced T lymphocyte proliferation (Houssiau et al, 1988, Kawakami et al, 1989) which involves the production of IL-2 and expression of the IL-2 receptor (Tosato and Pike, 1988, Tosato et al, 1990).

While in these models the cytokines IL-1 and IL-6 have co-stimulatory activity, in many systems the second signal must be delivered by direct contact between the T lymphocyte and the APC (Kawakami et al, 1989). The contact-dependent augmentation of T lymphocyte IL-2 production by endothelial cells is inhibited by antibodies against the CD2 molecule on the T lymphocyte or against lymphocyte function-associated antigen-3 (LFA-3) on the endothelial

cell (Hughes et al, 1990). LFA-3 is the ligand for the CD2 receptor (Dustin et al, 1987a), and this interaction will be discussed in more detail in section 4. Liposomes containing both purified LFA-3 and HLA-DR stimulate IL-2 production by T cell hybridomas, showing that CD2 binding to LFA-3 is sufficient to provide a co-stimulatory signal for those T cells (Bierer et al, 1988). There is evidence that the binding of CD2 to LFA-3 stimulates IL-1 release by the LFA-3-bearing cells (Le et al, 1990, Breitmeyer 1987). Endothelial cells express LFA-3 in vivo and in vitro (Springer et al, 1987).

Other accessory molecules on the surface of the endothelial cells and/or the T lymphocytes have been shown to have co-stimulatory roles in particular systems. These include intercellular adhesion molecule-1 (ICAM-1), CD44, and members of the very late antigen (VLA) family of molecules (or β_1 integrins). The functions of these molecules will be discussed in detail in sections 3 and 4.

Thus the precise identity of the co-stimulatory signal provided by endothelial cells remains unclear, but it appears to be multifactorial, with IL-1, IL-6, and activation by direct contact making significant contributions.

The evidence is convincing that cultured endothelial cells are fully competent APC, and it seems reasonable to extend this to endothelial cells in vivo, particularly in humans where they are normally HLA class II positive. However, unstimulated cultured endothelial cells are generally found to be HLA class II negative, but they become positive after stimulation with phytohaemagglutinin (PHA) (Pober and Gimbrone, 1982), medium conditioned by activated T lymphocytes (Pober et al, 1983a, Wagner et al, 1985b), and γ-IF, which is produced by activated T lymphocytes (Pober et al, 1983a, Collins et al, 1984, Pober et al, 1983b, Geppert and Lipsky, 1985). γ-IF also induces HLA class II expression by dermal fibroblasts and smooth muscle cells (Collins et al, 1984, Pober et al, 1983b, Geppert and Lipsky, 1985).

Cultured endothelial cells become HLA class II positive soon after co-culture with allogeneic lymphocytes (Pober et al, 1983a) or with lymphocytes and nominal antigen (Wagner et al, 1985b), and the endothelial cell antigen-presenting functions are inhibited by anti-HLA class II antibodies. The induction of MHC class II antigens by allogeneic lymphocytes in the dog is blocked by cyclosporine, probably because it inhibits γ-IF releases by the lymphocytes (Groenewegen et al, 1985). It may be that a small subpopulation of the cultured endothelial cells is HLA class II positive constitutively, and initiates antigen presentation, followed by the release of γ-IF and increased class II expression (Nunez et al, 1983). The possibility that a few dendritic cells

were contaminating these cultures was excluded by using multiply passaged endothelial cells. Alternatively, some of previously activated T lymphocytes added to the endothelial cell cultures might secrete sufficient γ-IF to induce the HLA class II expression.

The endothelium in rejection of a vascularised allograft

Sensitisation to a non-vascularised allograft appears to arise in the draining lymphoid tissue where host APC present donor antigen to host T lymphocytes. In contrast, sensitisation to a vascularised allograft (eg kidney) occurs within the graft itself (Pederson and Morris, 1970). The endothelial cells and the passenger leucocytes (dendritic cells) are all competent APC and therefore can contribute to the primary immune response. Even if the passenger leucocytes are removed, a vascularised graft will still be lost to rejection, suggesting that the endothelium alone is sufficient to initiate primary recognition. It presents a large and accessible target for the alloimmune response as it expresses the polymorphic antigens that the recipient's immune system recognise as foreign. Understandably, endothelial damage is a major feature of the allograft rejection.

The effector mechanisms responsible for rejection can be broadly divided into antibody- and cell-mediated (Mason and Morris, 1986). Antibodies against donor antigens can develop as a result of previous transplantation, blood transfusion, or pregnancy. If the recipient possesses such antibodies before a transplant (ie is "sensitised" to the donor antigens), "hyperacute rejection" will result (Kissmeyer-Nielson et al, 1966).This is characterised by the binding of antibody and complement to the graft endothelium with consequent destruction mediated by polymorphonuclear leucocytes. This form of rejection is now very uncommon due to the avoidance of transplantation in recipients with positive lymphocyte crossmatches with the donor.

The allograft rejection that is now most commonly encountered in clinical medicine is probably both antibody- and cell-mediated. Antibodies still contribute to the vascular damage, and patients almost invariably develop anti-HLA antibodies following the loss of an allograft to rejection.

The alloimmune response that results in this form of rejection is primarily under the control of the cellular immune system, which is evidenced by the infiltration of mononuclear cells into rejecting allografts. However, there is disagreement about whether it is primarily a helper T lymphocyte-mediated delayed-type hypersensitivity response or is a result of cytotoxic T lymphocyte

lysis of the target cells (Loveland and McKenzie, 1982, Hall and Dorsch, 1984a, Hayry 1984, Mason and Morris, 1986). A typical cellular infiltrate in a rejecting kidney contains 30 to 35% T lymphocytes, with usually more CD8-positive than CD4-positive cells. This ratio is approximately the same in stable grafts (Mason and Morris, 1986). Monocytes/macrophages make up about 60% of the infiltrate, and natural killer (NK) cells less than 10%. The lymphocytic infiltrate is enriched for antigen specific cells (Scheper et al, 1985, Modlin et al, 1988, Tilney et al, 1978), but the inflammatory reaction also stimulates the migration of non-antigen specific cells into the tissue (Hopt et al, 1983, Hall and Dorsch, 1984a).

Microvascular destruction is a prominent early feature of rejection of a vascularised allograft (Dvorak et al, 1979, Bishop et al, 1989b), and it precedes the damage to other tissue components (Forbes et al, 1983). The endothelial cells initially develop a characteristic plump, hypertrophied appearance and separate from each other (Pederson and Morris, 1970, Forbes et al, 1983). This is reminiscent of the changes they undergo at sites of chronic inflammation (Graham and Shannon, 1972), and appears to precede (and allow) the efflux of effector cells into the graft. The major site of extravasation of cells into a renal allograft is the peritubular capillaries (Renkonen et al, 1989b, Bender et al, 1989). The changes in appearance of the vessels is probably produced by cytokines released by the activated effector cells, and represents a state of activation of the endothelium. This will be discussed in more detail in section 4.

Antibodies against the endothelium in autoimmune disease

The immune response to an allograft is an example of the appropriate reaction to foreign antigen, and the role of the endothelium in this process is the primary topic of this section of the thesis. However, it is also instructive to briefly examine the role of the endothelium in autoimmune disease, where the immune system turns against self-antigens and causes unwanted tissue damage. Vasculitis is a prominent feature of a number of autoimmune conditions, demonstrating that the endothelium has become a major target of the inappropriate immune response. In many such cases, antibodies directed against endothelial cells are detectable in the patient's serum. The autoantigens recognised by these anti-endothelial cell antibodies (AECA) have not been identified, and it is also uncertain whether the autoantibodies

make a significant contribution to the vasculitis or are just markers of endothelial damage.

Several studies have reported AECA in patients with systemic vasculitis. Cerilli et al (1987) found autologous anti-monocyte antibodies in 22 out of 30 patients (73%) with necrotising vasculitis. Twenty of these positive sera also reacted with cultured HUVEC but not lymphocytes, and so were thought to be specific for the EM system. They also found that 31 out of 48 patients (65%) with angiographically confirmed coronary artery disease had anti-EM antibodies. In a more recent study, the same group found cytotoxic, complement-fixing AECA in 18 out of 21 patients (86%) with systemic vasculitis (not of the hypersensitivity type) (Brasile et al, 1989). Ferraro et al (1990) found AECA in 9 out of 15 patients with micropolyarteritis and in 2 out of 5 patients with Wegener's granulomatosis, although these sera did not lyse cultured HUVEC, with or without complement.

A number of studies have examined AECA in systemic lupus erythematosus (SLE) and other connective tissue disorders. Cines et al (1984) detected complement-fixing AECA in the sera of patients with active SLE using a solid phase radioimmunoassay. Penning et al (1984, 1985) found cytotoxic activity for cultured HUVEC (when co-cultured with human peripheral blood mononuclear cells) in the sera of 3 out of 35 patients with SLE and 9 out of 39 patients with progressive systemic sclerosis (PSS). Holt et al (1989) found the same in 10 out of 48 patients with PSS. Hashemi et al (1987) detected IgG and IgM AECA by ELISA in patients with SLE and PSS, and showed that the binding was not mediated by Fc receptors. Rosenbaum et al (1988) also used an ELISA to show IgG AECA in 74% of patients with SLE, in 30% with PSS, and in 28% with rheumatoid arthritis (RA). The antibodies were apparently unrelated to other circulating autoantibodies, including anti-cardiolipin or anti-DNA IgG. The AECA in all the sera was absorbed out by culture with dermal fibroblasts. Vismara et al (1988) found AECA in 39.2% (IgG) and 45.1% (IgM) of sera from SLE patients, with no correlation with clinical or laboratory parameters of disease activity, including anti-cardiolipin antibodies. However, they showed that affinity-purified anti-cardiolipin antibodies reacted with cultured HUVEC, and suggested that some AECA are directed against negatively charged phospholipids on the surface of the endothelial cells. Heurkens et al (1989) detected IgG AECA in 19 out of 28 patients with rheumatoid vasculitis, 4 out of 24 with RA, and 7 out of 10 with SLE. The titre of AECA in 4 of their patients with rheumatoid vasculitis

correlated with the clinical course. The AECA activity was also absorbed out by fibroblasts.

AECA have also been detected in a variety of other diseases, including 32% of patients with IgA nephropathy (Yap et al, 1988), IgM AECA in 6 patients with autoimmune hypoparathyroidism (Fattorossi et al, 1988), and complement-fixing IgG and IgM AECA in 13 out of 14 children with acute haemolytic-uraemic syndrome plus 3 out of 5 adult patients with acute, non-relapsing thrombotic thrombocytopenic purpura (Leung et al, 1988).

Leung et al (1988) have also performed a series of studies on patients with Kawasaki syndrome, an acute febrile illness of early childhood, characterised by diffuse vasculitis and marked T and B lymphocyte activation. Serum IgM antibodies were found in the acute phase of the illness that specifically lyse HUVEC after they have been stimulated with γ-IF (Leung et al, 1986a), IL-1, or TNF (Leung et al, 1986b). They postulated that activated monocytes/macrophages or other cells release cytokines which predispose the endothelium to injury by the circulating antibodies. Ten out of 16 patients had peripheral blood mononuclear cells secreting high levels of IL-1 (Leung et al, 1989), and two endothelial activation markers, ELAM-1 and ICAM-1 (see section 4) were detected by indirect immunoperoxidase staining on the endothelium of skin samples taken from 5 patients. These activation markers are upregulated on cultured HUVEC by γ-IF (ICAM-1 only), IL-1, and TNF.

However, despite finding AECA in a number of conditions, there are still a numbered of unanswered questions about their significance. No group has convincingly elucidated the structure of any of the putative antigens recognised by the AECA. This may be because they are carbohydrate or phospholipid determinants that are more difficult to identify, or due to low affinity of the AECA. In addition, the AECA usually correlate poorly, if at all, with the clinical course and laboratory markers of disease activity. One concern is that much of the AECA activity may be due to non-specific binding of the antibodies to the HUVEC and fibroblasts. In most of the diseases where AECA have been detected, there is a general overproduction of antibodies directed against a myriad of determinants, plus circulating immune complexes. It remains to be proven that AECA have a role in the pathogenesis of these diseases. The studies of Leung et al into Kawasaki syndrome possibly have found the most convincing examples of AECA that are relevant to a disease process.

2.2 Studies of the expression of HLA class I and class II molecules in the normal human kidney and in rejecting renal allografts

Expression of HLA class I antigens in the normal human kidney

The two murine anti-HLA class I monoclonal antibodies used in these studies (W6/32 and RM7.30) both recognise monomorphic determinants on all class I molecules. Normal kidney sections were cut from biopsies taken from the tumour-free portion of two kidneys removed surgically for renal cell carcinoma, and from wedge biopsies taken from kidneys just prior to their use as allografts. The latter kidneys had been perfused and stored at 4°C for 12 to 24 hours. The morphology of these sections was minimally abnormal in some cases (mainly tubular changes), but the results of staining with the anti-HLA class I antibodies were no different between the tissues from the two sources.

Each of the antibodies gave identical staining patterns (see photograph page 66). HLA class I is strongly expressed on glomerular capillaries and intertubular capillaries. Veins are weakly positive, and arterioles and arteries range from negative to weakly positive. In biopsies examined from 13 different patients, 3 definitely and 1 possibly had weak staining of the luminal aspect of the proximal tubules. The other structures in the kidney were consistently negative.

Expression of HLA class II antigens in the normal human kidney

Four anti-HLA class II murine monoclonal antibodies were used in these studies. RM5.112 binds to a monomorphic determinant on all class II molecules, L243 binds to HLA-DR molecules, Genox 3.53 to HLA-DQw1, and B7/21 to HLA-DP. HLA-DQw1 is limited to those individuals who express HLA-DR1,-2,-6,-8, or -10, which is about 75% of Caucosoids, the ethnic group from whom these biopsies were taken.

HLA class II antigens are moderately strongly expressed on glomerular and intertubular capillaries, and weakly on the larger vessels (see photograph page 66). In general, the staining is slightly weaker than HLA class I. HLA-DR makes up most of the class II expression, while HLA-DQw1 and HLA-DP are usually quite weak, particularly on glomerular capillaries. Three out of the 13 specimens examined were weakly HLA class II positive on the luminal aspect of the proximal tubules.

Expression of HLA class I antigens in rejecting renal allografts

The morphology of the biopsies taken from rejecting allografts was less well preserved than that in the normal kidneys. The intertubular capillaries are frequently almost indistinguishable due to the damage caused by the rejection process, and the infiltrating leucocytes often obscure the underlying anatomy. However, it is still possible to make a number of conclusions from studying these biopsies, particularly when a particular antigen is lacking or only weakly expressed in normal kidneys.

Six patients were studied in whom biopsies were taken from the transplanted kidney before transplantation and at a later date during an episode of rejection. The endothelial expression of HLA class I antigens appears to be slightly increased during rejection, but the high basal expression obscures clearcut changes. In contrast, there is often dramatic staining of the tubular cytoplasm during rejection, which is clearly greater than the proximal tubule staining in some normal kidneys (see photograph page 67). The infitrating leucocytes also strongly express HLA class I.

Two other patients had biopsies taken before transplantation and later during an episode of allograft dysfunction that proved to be due to cyclosporine toxicity rather than rejection. In these cases, there were no clear changes in HLA class I expression on the endothelium or the tubules.

Expression of HLA class II antigens in rejecting renal allografts

Endothelial staining with the anti-HLA class II antibodies is more clearly increased during rejection than with anti-HLA class I antibodies (see photograph page 67). This is more easily recognised because of the lower expression of HLA class II compared with HLA class I in normal kidneys. The increased expression is most apparent in the large vessels. Again, HLA-DR accounts for most of the staining, but HLA-DP is usually definitely stronger than in the normal kidneys. However, HLA-DQw1 shows very little change. Many of the infiltrating cells are also HLA class II positive.

The most clearcut change is the often de novo appearance of HLA class II on the tubular cytoplasm, which is almost exclusively due to HLA-DR (see photograph page 67). Eleven patients who had biopsies taken before transplantation and at a later date during an episode of allograft dysfunction were studied. There was no tubular expression of HLA class II in any of these pretransplantation biopsies. Of the 12 biopsies taken after transplantation (one

patient was biopsied twice), 8 showed evidence of rejection and in 7 of these the tubules were HLA class II positive. The remaining 4 biopsies showed no evidence of rejection and had no tubular staining for HLA class II (see table 1, page 72).

This apparent specificity of tubular HLA class II expression for rejection was tested on a series of randomly selected biopsies from different patients (see table 2, page 72). Seven biopsies were taken from allografts within 1 hour of establishment of vascular anastomosis, and in 3 of these the tubules expressed HLA class II. None of the 7 biopsies showed any evidence of hyperacute rejection. Sixteen biopsies taken during episodes of acute allograft dysfunction were stained with the anti-HLA class II antibody RM5.112. Eight of these had acute rejection, and in all 8 the tubules were positive. The other 8 had no rejection, but in 4 of these the tubules were also positive. Hence a number of non-rejecting kidneys also expressed tubular HLA class II, albeit generally weaker than during rejection.

2.3 Studies of the expression of HLA class I and class II molecules in normal skeletal muscle and peripheral nerves, and in vasculitis of the peripheral nervous system

Expression of HLA class I and class II antigens in skeletal muscle and peripheral nerves

In a collaborative study with Dr. P. Panegyres from the Neurology Department at The Queen Elizabeth Hospital, specimens of human skeletal muscle and peripheral nerve were stained with monoclonal antibodies against HLA class I (W6/32) and class II (RM5.112).

Three muscle specimens had a normal microscopic appearance. One had been taken from a normal individual, one patient had a non-inflammatory myopathy, and the third was in remission from vasculitis. W6/32 strongly stains the capillaries between individual muscle fibres and the intima of larger vessels. There is weak to moderate staining of the media of large vessels, and some variable weak staining around the periphery of individual muscle fibres (see photograph page 68). The perimysium that surrounds each muscle fasciculus also stains strongly. RM5.112 only stains the capillaries between fibres and the intima of larger vessels (see photograph page 68).

Sections from a nerve biopsy taken from a patient with a non-inflammatory peripheral neuropathy (tomaculous neuropathy) were also stained. In the absence of a biopsy from a patient with completely normal nerves, this was regarded as the normal control for the purposes of this study. W6/32 strongly stains the capillaries, intima of large vessels, and the endoneurium surrounding individual nerve fibres, and weakly stains the media of large vessels. There is very weak staining of the perineurium around each nerve fasciculus and connective tissue around vessels (see photograph page 69). RM5.112 moderately stains the capillaries and intima of large vessels, the endoneurium weakly, and the perineurium very weakly (see photograph page 69).

Expression of HLA class I and class II antigens in vasculitis of the peripheral nervous system

Biopsies from 7 patients (4 muscle and 3 nerve) with vasculitis of the peripheral nervous system were stained with W6/32 and RM5.112. The patient

with the most florid vasculitis out of the muscle biopsies also had had a biopsy taken during remission (see above), allowing direct comparison of expression of the HLA antigens at these different time points in the disease process. W6/32 strongly stained the following structures: capillaries between muscle fibres, large vessels (including media), perimysium, and infiltrating cells. The periphery of muscle fibres was more clearly stained, and the fibres themselves were definitely weakly to moderately positive (see photograph page 70). RM5.112 strongly stained the capillaries between fibres and the periphery of the fibres, but not their cytoplasm.The intima of large vessels also strongly stained, and there was moderate staining of the perimysium and infiltrating cells (see photograph page 70). The clearest changes from the remission biopsy were the increased staining with both antibodies at the periphery of the fibres and the appearance of HLA class I in their cytoplasm. There is also a probable increase in endothelial staining, but the moderate to high basal expression makes it difficult to be certain. The pattern of staining was comparable in the other muscle biopsies, and the strength of staining seemed to correlate with the degree of inflammation.

In the inflamed nerve biopsies, there was increased staining with W6/32 of the intima, media and surrounding connective tissue of the large vessels, and probable increase on the endoneurium (see photograph page 71). RM5.112 was stronger on the intima of large vessels, and clearly increased on the endoneurium (see photograph page 71). The infiltrating cells stained strongly with both antibodies.

Discussion of sections 2.2 and 2.3

The HLA class I and class II antigens have very similar distributions in the normal kidney, and are predominantly found on the endothelium of glomerular and intertubular capillaries. HLA class I is found more frequently on the tubular cells (in low amounts), and is consistently stronger on larger vessels. The class II expression is predominantly composed of HLA-DR molecules, with HLA-DQw1 and HLA-DP making minor and approximately equal contributions.

There have been a number of studies of the distribution of HLA antigens in kidneys and other organs. Hancock et al (1982) localised HLA class I to all renal endothelium, Bowman's capsule, and consistently on the proximal tubules (diffusely scattered throughout the cytoplasm), and HLA-DR to capillary and venous endothelium and weakly on the proximal tubules. Hart et

al (1981c) and Fuggle et al (1983) found that anti-HLA class I antibodies intensely stained the glomeruli and intertubular areas (including dendritic cells), and diffusely stained the tubules. HLA-DR was found on glomerular mesangium, tubular cells, dendritic cells, and in low levels on all endothelium (although stronger on the capillaries than the large vessels). In a later study, the same group (Fuggle et al, 1987) found that HLA-DR comprised all the class II antigen on the tubules of normal kidneys. Halloran et al (1986) showed that HLA class I was strongly expressed on all endothelium, weakly on proximal tubules, and was equivocal on other structures. HLA-DR was weak or negative on proximal tubules, and positive on all endothelium apart from the arteries and afferent arterioles. HLA-DQ was negative. Evans et al (1984, 1985a) found variable expression of HLA-DR, particularly on the proximal tubules, and that the arteries were consistently negative. HLA-DQ and HLA-DP were variably detected on all endothelium, but were most consistently found on the intertubular capillaries and least often on the arteries (Evans et al, 1985b).

The summary of these studies is that HLA class I has been described on all cells, although there is disagreement about the mesangium and Bowman's capsule. HLA class II is found on all endothelium apart from arteries and arterioles, and intermittently on the proximal tubules. Studies in the rat have strongly suggested that the tubular class II is produced by the cells themselves rather than being non-specifically absorbed onto the cells (Hart et al, 1981b, Mayrhofer and Schon-Hegrad, 1983). The studies in this thesis have shown less frequent expression on the tubules, but otherwise are in agreement with the other investigations. The differences may be due to the staining techniques or sampling. The kidneys in this thesis were gemerally fixed with PLP, whereas most other studies have studied frozen, acetone-fixed samples.

The studies in this thesis have shown that both HLA class I and class II antigens are induced on the endothelium and the tubular cells during renal allograft rejection. The most clearcut change on the endothelium is the increase in HLA class II on arteries, which is in agreement with the findings of Halloran et al (1986). The increase in tubular HLA antigens is consistent with a number of other studies. The first documentation of tubular HLA class II expression during renal allograft rejection was by Hall et al (1984b), who found that tubular HLA-DR increased significantly in 25 consecutive cases with severe cellular rejection, but in only 4 out of 14 with no evidence or minimum evidence only of rejection. HLA-DR was also increased on tubular cells aspirated from kidneys undergoing acute rejection. Fuggle et al (1986)

also showed that the tubular HLA class II expression correlated with cellular infiltrate and rejection, and later that it was made up of HLA-DR, -DQ, and -DP (Fuggle et al, 1987). Specific polymorphic antibodies proved that the induced antigen was of donor origin.

The studies described here again show that tubular HLA class II expression correlates with acute rejection. However, HLA class II molecules can also be present on the tubules in allografts not undergoing acute rejection, albeit usually weaker. In addition, they have been found in chronic rejection (Toma et al, 1987) and in a number of other kidney disorders, especially where there is a cellular infiltrate (Wadgymar et al, 1987, Muller et al, 1989). In the appropriate clinical setting, strong HLA class II expression by the tubular cells can correlate with acute rejection. Staining tubular cells obtained by aspiration of the allograft or from the urine with anti-HLA class II antibodies may be of clinical value.

The damaged intertubular capillaries are difficult to assess during rejection, but some groups have claimed that they express decreased amounts of HLA antigens in this setting (Halloran et al, 1986, Bishop et al, 1989b). It is unclear whether this is a real decrease, or just appears less because of the local injury.

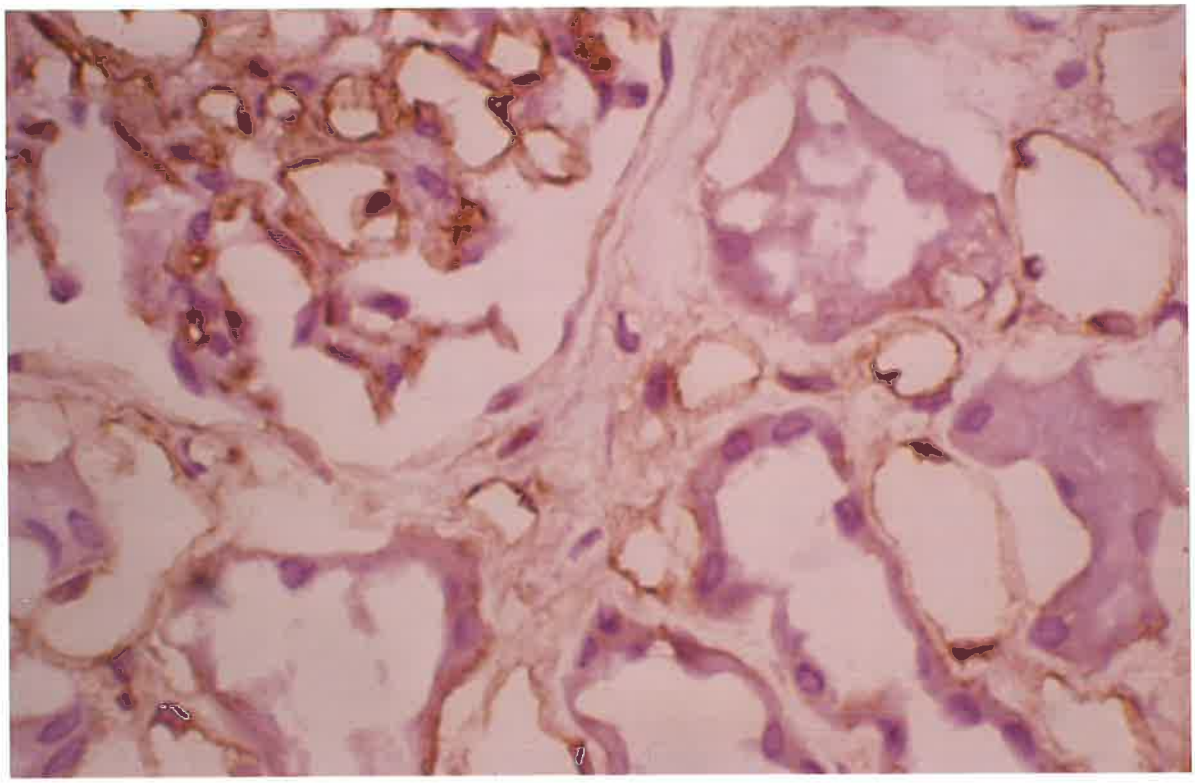
The induction of MHC antigens in inflammation is not confined to the human kidney. It appears that the inflamed endothelium in vasculitis involving skeletal muscle and peripheral nerves expresses increased amounts of HLA molecules, and there is new expression of HLA class I on the muscle fibres and a dramatic increase in the expression of HLA class II by the endoneurium that surrounds individual nerve fibres. Appleyard et al (1985) have also found HLA class I on muscle fibres in patients with myositis and various X-linked muscular dystrophies.

A number of studies have looked at MHC induction in other tissues. HLA-DQ and -DP are induced on the endothelium in proportion to the cellular infiltrate in ileum affected by Crohn's disease (Koretz et al, 1987). Induction of HLA antigens on epithelial cells occurs in heart transplants (Rose et al, 1986), graft-vs-host disease (on keratincytes) (Sviland et al, 1988, Lampert et al, 1981), polymyositis (Rowe et al, 1983), Hashimoto's thyroiditis and Grave's disease (on thyrocytes) (Hanafusa et al, 1983), herpes simplex oesophagitis (Geboes et al, 1985), and in a number of inflammatory bowel disorders (Bland, 1988).

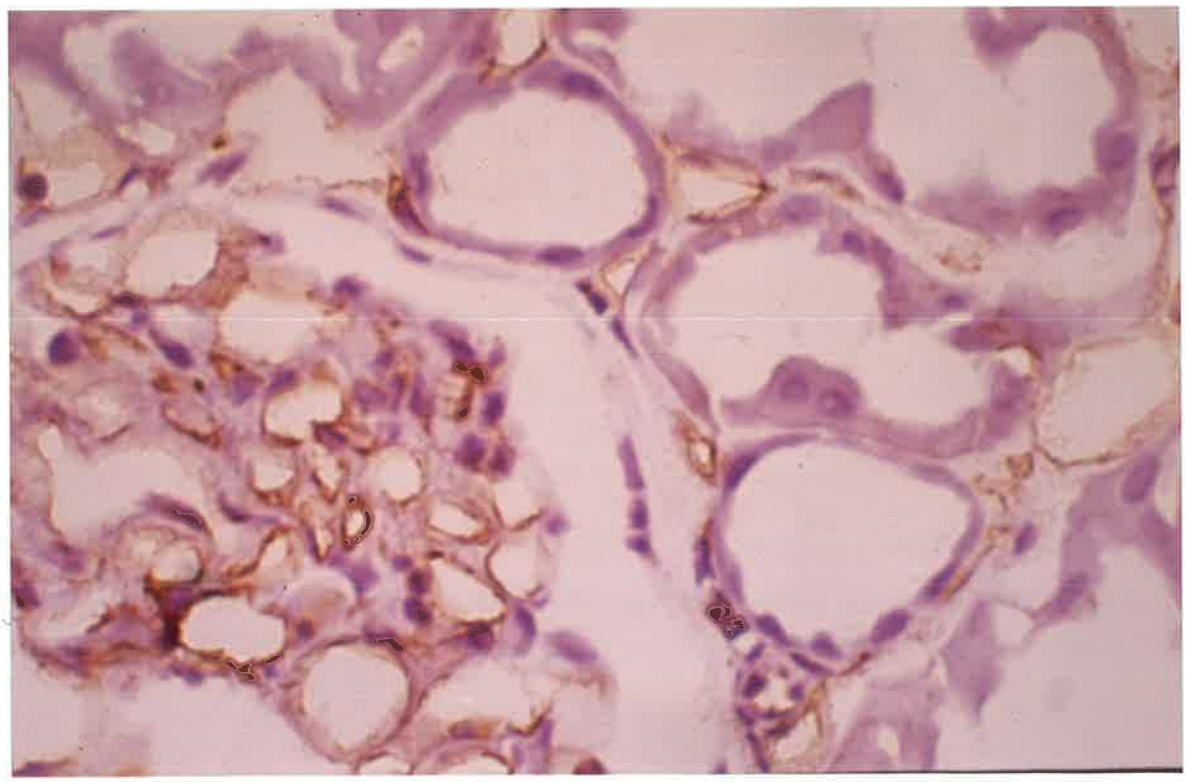
In the mouse, induction of Ia antigens on endothelium occurs in virus-induced demyelination (Rodriguez et al, 1987) and rejecting skin allografts (de Waal et al, 1983).

The upregulation of MHC antigens in these various disorders is probably induced by cytokines produced by activated cells at the site of inflammation. γ-IF is likely to be a major mediator of this process. It induces class I and class II antigens on cultured endothelial cells (see literature review for this section), uveal cells (Abi-Hanna and Wakefield, 1989), keratinocytes (Czernielewsk and Bagot, 1986), renal tubular cells (Bishop et al, 1986, Bishop et al, 1988), and thyroid cells (Weetman and Rees, 1988). γ-IF injected into baboons (Munro et al, 1989) and mice (Skoskiewicz et al, 1985) also increases MHC expression. α-IF, TNF, and LPS, which increase class I expression and have synergistic effects with γ-IF, probably also contribute to the upregulation in vivo (Weetman and Rees, 1988, Munro et al, 1989, Jephthah-Ochola et al, 1988). The increased MHC expression usually correlates with the degree of cellular infiltrate, although in experimental allergic encephalomyelitis in mice, the endothelial Ia increases prior to the the influx of leucocytes (Sobels et al, 1984). This suggests that the increased MHC may be an initiator of the inflammation rather than a simple consequence of it.

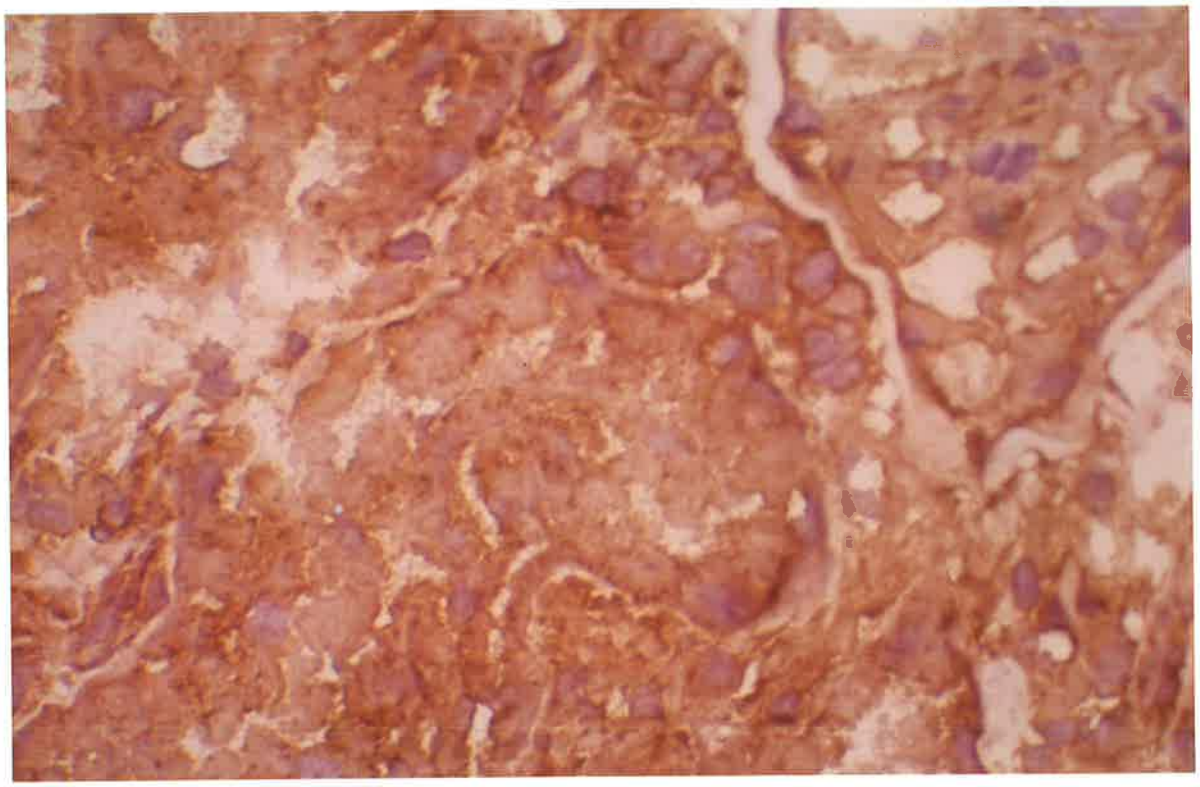
The upregulation of HLA has a number of potential consequences in the context of allograft rejection. It presumably renders cells more "immunogenic" (by increasing the number of potential targets for the host effector cells), and enhances any antigen presenting function (which is particularly relevant to the endothelium). Significantly, a group of patients given α-IF as prophylactic antiviral therapy following renal transplantation had a high incidence of rejection, possibly secondary to induction of HLA class I antigens in the graft (Kramer et al, 1984). In addition, one possible explanation for the initiation of autoimmune disease is viral infection-induced aberrant expression of class II antigens on the cells that are the targets of the autoantibodies (Bottazzo et al, 1983).



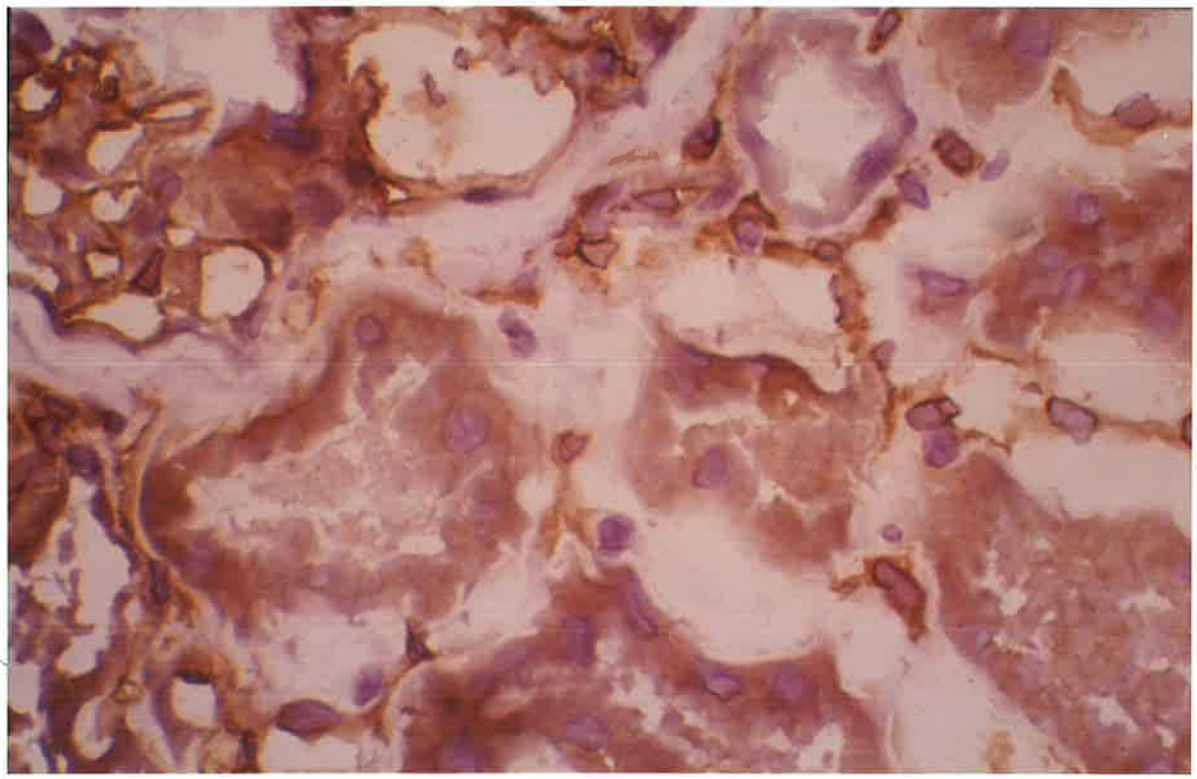
HLA class I (W6/32) expression in normal kidney (indirect immunoperoxidase, x400)



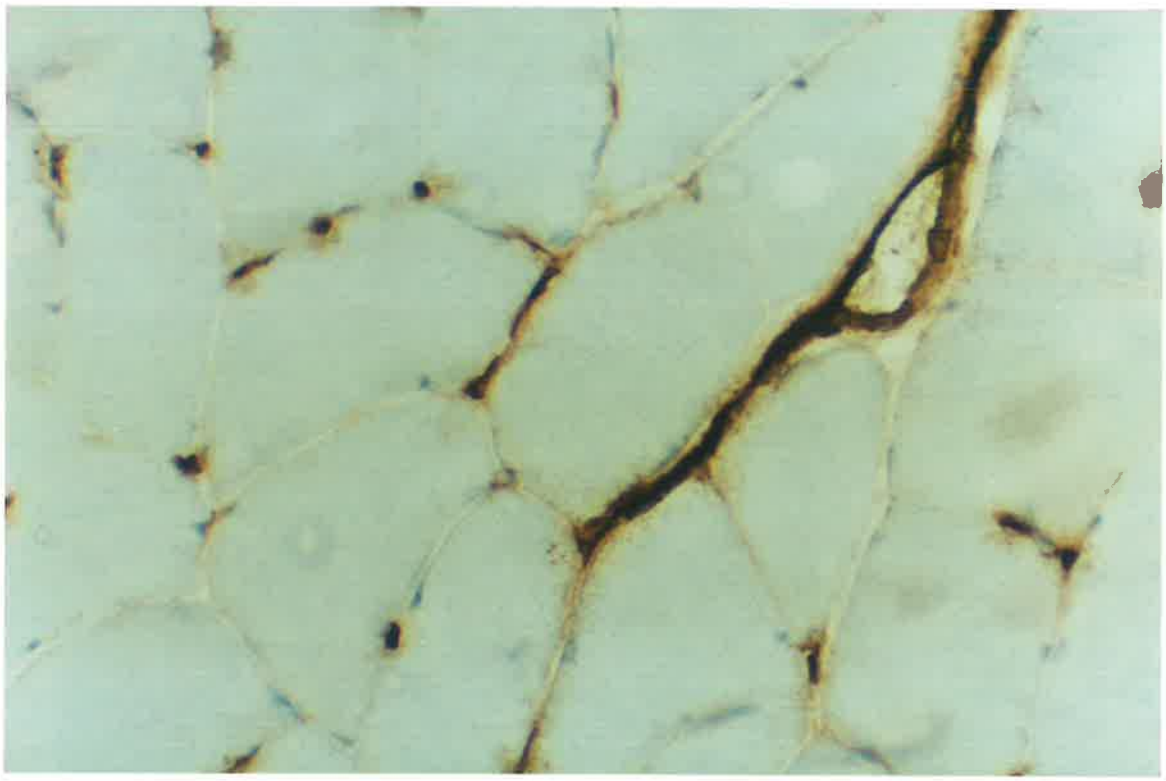
HLA class II (RM5.112) expression in normal kidney (indirect immunoperoxidase, x400)



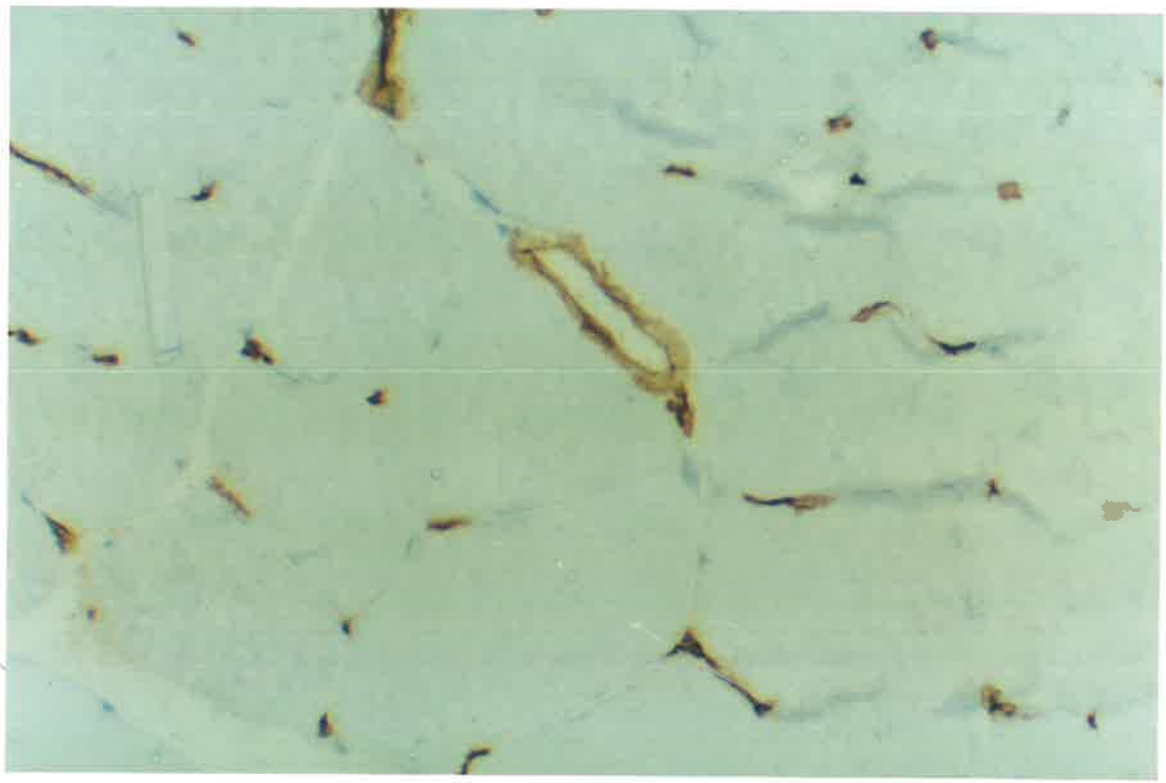
HLA class I (W6/32) expression in rejecting renal allograft (indirect immunoperoxidase, x400)



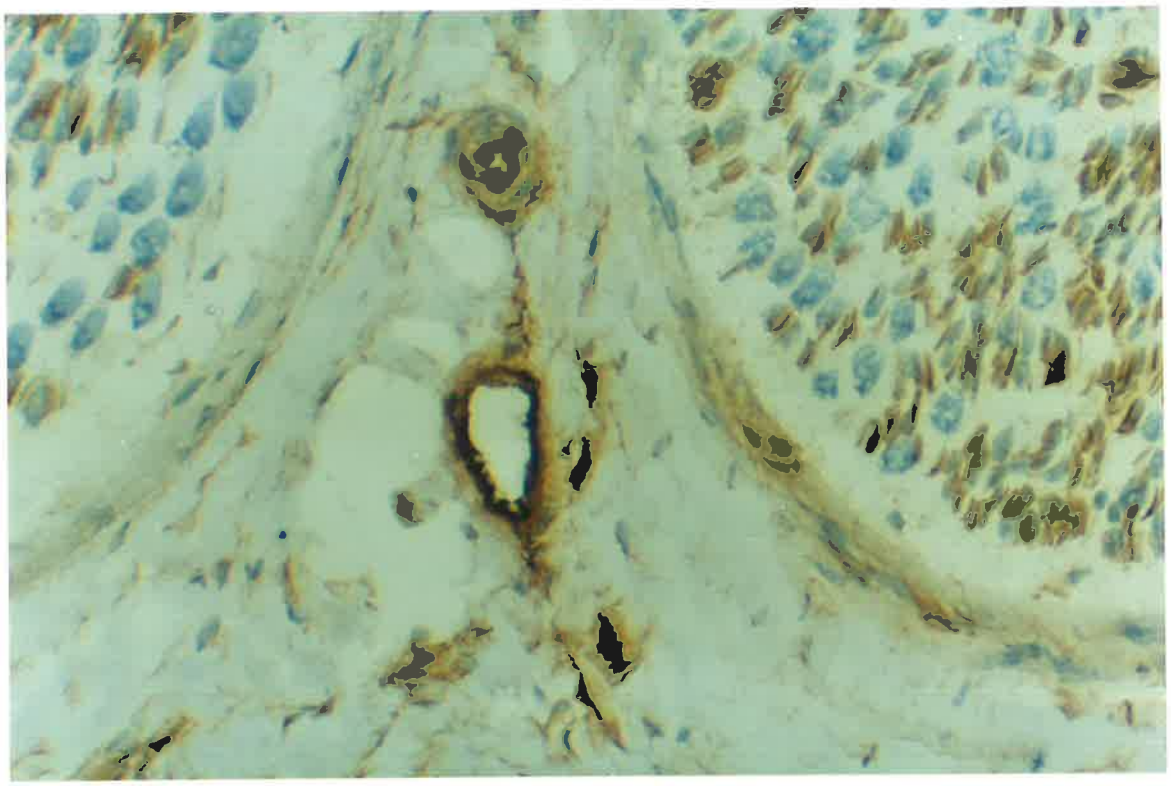
HLA class II (RM5.112) expression in rejecting renal allograft (indirect immunoperoxidase, x400)



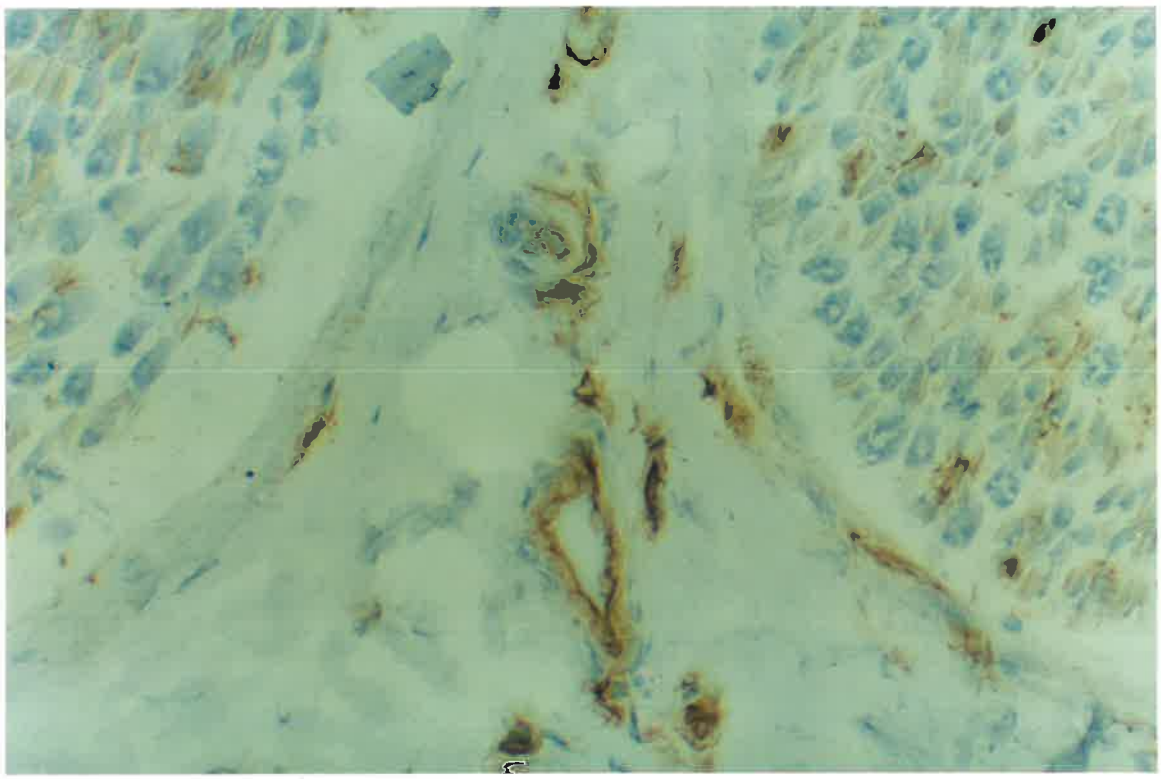
HLA class I (W6/32) expression in normal skeletal muscle (indirect immunoperoxidase, x200)



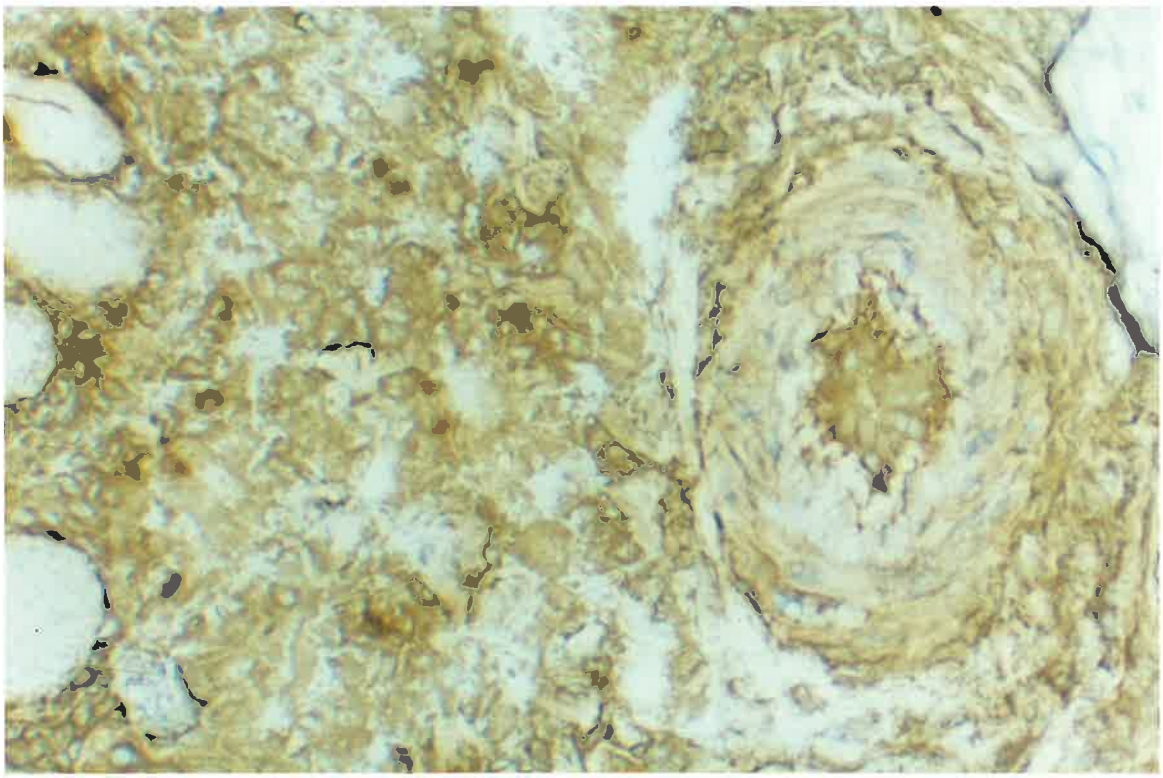
HLA class II (RM5.112) expression in normal skeletal muscle (indirect immunoperoxidase, x200)



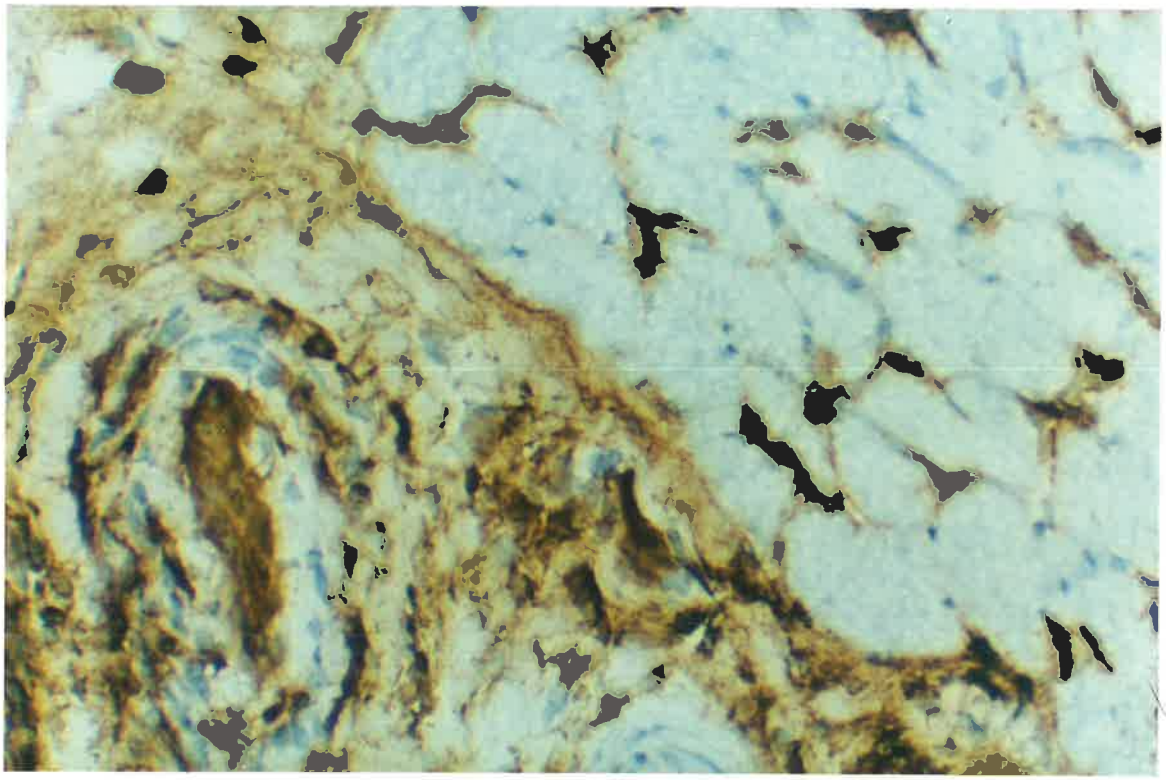
HLA class I (W6/32) expression in peripheral nerve (tomaculous neuropathy) (indirect immunoperoxidase, x200)



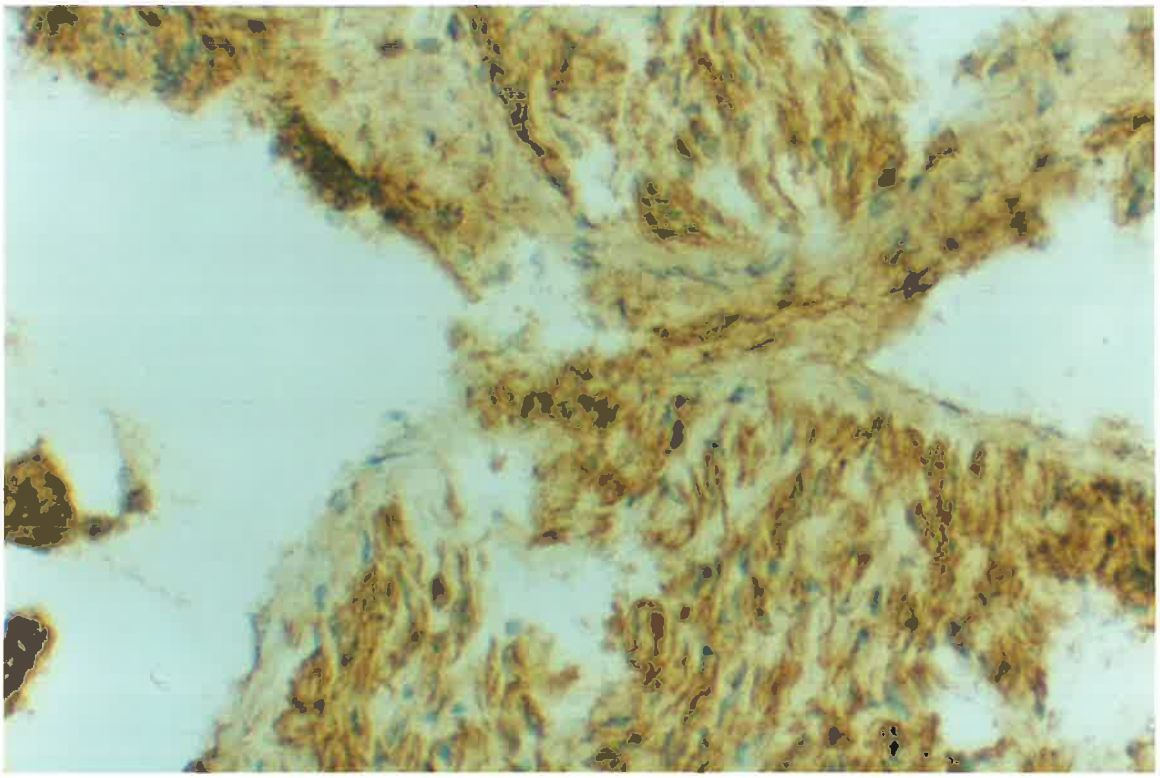
HLA class II (RM5.112) expression in peripheral nerve (tomaculous neuropathy) (indirect immunoperoxidase, x200)



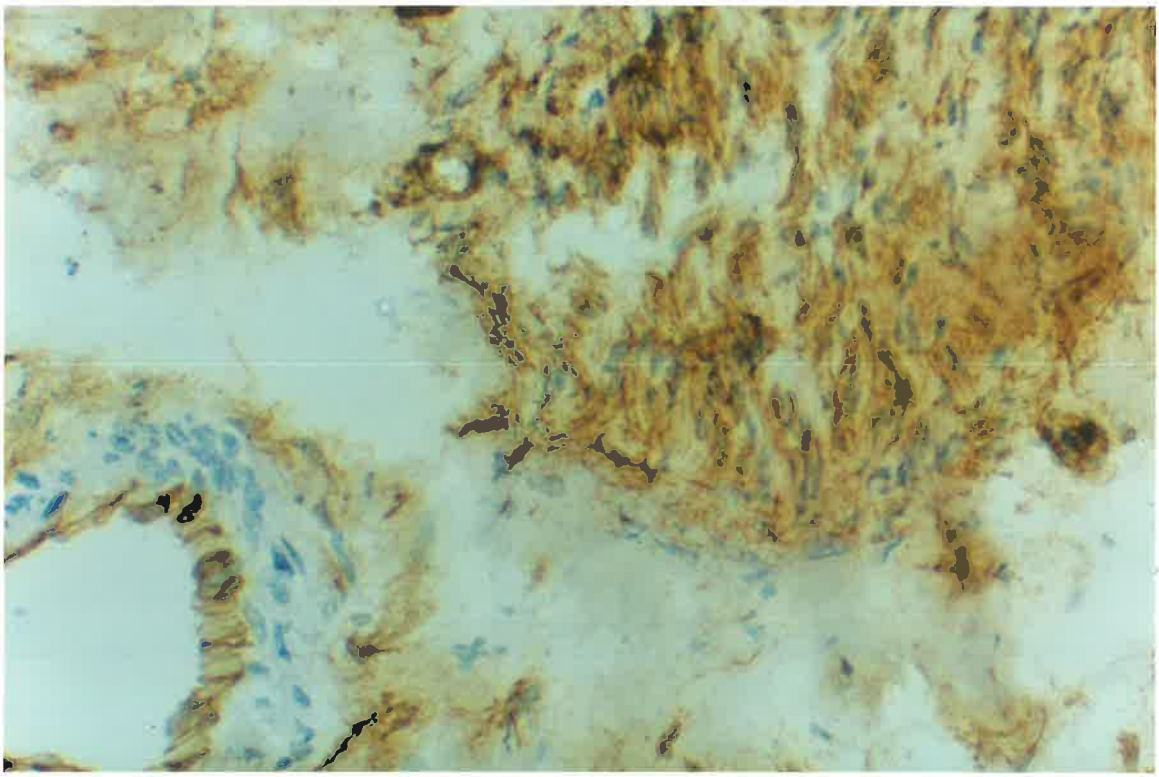
HLA class I (W6/32) expression in vasculitis involving skeletal muscle
(indirect immunoperoxidase, x200)



HLA class II (RM5.112) expression in vasculitis involving skeletal muscle
(indirect immunoperoxidase, x200)



HLA class I (W6/32) expression in vasculitis involving a peripheral nerve (indirect immunoperoxidase, x200)



HLA class II (RM5.112) expression in vasculitis involving a peripheral nerve (indirect immunoperoxidase, x200)

TABLE 1: Expression of HLA class II molecules and ICAM-1 on tubular cells of paired biopsies of renal allografts taken before and after transplantation.

	HLA class II	ICAM-1
Pretransplantation (n = 11)	0	2[a]
Posttransplantation		
Rejection (n = 8)	7	7
No rejection (n = 4)	0	0

[a] ICAM-1 was expressed on tubules in 2 pretransplant biopsies; the later 2 biopsies of these kidneys showed neither rejection nor tubular ICAM-1 expression.

TABLE 2: Expression of HLA class II molecules and ICAM-1 on tubular cells of random biopsies of renal allografts; postanastomosis biopsies were taken within 60 minutes of initial vascularisation, and later biopsies were taken at the time of acute allograft dysfunction at between 1 and 36 days posttransplantation.

	HLA class II	ICAM-1
Postanastomosis biopsies (n = 7)	3	3[a]
Later biopsies		
Rejection (n = 8)	8	7
No rejection (n = 8)	4	2[b]

[a] The same 3 biopsies showed HLA class II and ICAM-1 expression.
[b] The 2 later biopsies of nonrejecting transplants that were positive for tubular ICAM-1 also expressed HLA class II molecules.

SECTION 3:
ENDOTHELIAL CELL EXTRACELLULAR MATRIX RECEPTORS

3.1 Literature Review

Introduction

The extracellular matrix (ECM) is comprised of a complex mesh of macromolecules that surrounds cells and provides support for the tissues. The two main classes of macromolecules that make up the ECM are the polysaccharide glycosaminoglycans (that are usually covalently linked to a core protein to form proteoglycans), and fibrous proteins, the most abundant of which are collagen, elastin, fibronectin and laminin. The fibrous proteins are embedded in a hydrated gel formed by the glycosaminoglycan and proteoglycan molecules. Cells (particularly fibroblasts in the connective tissue) secrete the ECM components locally, and attach to them via specialised adhesion receptors. These adhesion molecules are the subject of this section.

Extracellular Matrix Components

Proteoglycans

Glycosaminoglycans are long, unbranched polysaccharide chains composed of repeating disaccharide units. There are four main groups, of which all but the first are linked to protein:

(1) hyaluronic acid, the largest and simplest
(2) chondroitin sulphate and dermatan sulphate
(3) heparan sulphate and heparin, and
(4) keratan sulphate.

The glycosaminoglycans can bind to other ECM molecules (eg fibronectin), and the strength of this binding is proportional to the degree of sulphation of the glycosaminoglycan (Ruoslahti, 1989) Heparin is the most highly sulphated and so binds most strongly. Proteoglycans are also found on cell surfaces and can mediate cell adhesion to ECM proteins. For example, syndecan is a proteoglycan with heparin and chondroitin sulphate side chains. It mediates adhesion to interstitial matrix components by binding to fibronectin and to interstitial collagens (types I, III, and V), but but does bind to the basement membrane components laminin and type IV collagen (Saunders and Bernfield, 1988). The cell surface proteoglycans probably have an auxillary anchoring role that complements the more specific integrin-mediated adhesion (see later) (Ruoslahti, 1989). This is consistent with the observation that cell surface proteoglycan associates with the actin-rich cytoskeleton in the

process of cells forming stable bonds to the ECM (Rapraeger et al, 1986). In addition, the luminal surface of vascular endothelial cells bears proteoglycans containing antithrombogenic heparan sulphate chains that significantly contribute to the nonthrombotic properties of the vessel wall (Marcum and Rosenberg, 1989).

Proteoglycans, mainly with heparan sulphate side chains, can form polyanionic sites which have been implicated in the ionic control of filtration through glomerular basement membranes (Timpl, 1989).

Collagen

The fibrous protein collagen is the most abundant protein in the ECM. The fibres have a characteristic triple-stranded helical structure, and are extremely rich in proline and glycine. About 10 different collagen molecules have been described but the best characterised are types I, II, and III (the fibrillar collagens), and type IV. Types I, II, and III are the main collagens found in connective tissue (type I is the most common), and they form high tensile collagen fibres. Type IV collagen is confined to and makes up the core of basement membranes, and forms a sheet-like meshwork rather than fibrils (Timpl, 1989). Endothelial cells can synthesise collagen (Sage et al, 1981), and cultured endothelial cells will preferentially adhere to and migrate on type IV collagen (Herbst et al, 1988). Cell lines in culture will reorganise and resemble the morphology of the original tissue when grown in three-dimensional collagen gels (Hall et al, 1982).

Fibronectin

Fibronectin is a large fibril-forming glycoprotein that has multiple roles in adhesion (Mosher,1984). It exists in three forms:

(1) plasma fibronectin, a soluble dimer implicated in coagulation, wound healing, and phagocytosis (opsonisation),

(2) cell surface fibronectin oligomer, and

(3) matrix fibronectin, forming highly insoluble fibrils.

The basic subunit (molecular weight 220 kD) has multiple domains with different functions. One domain binds to collagen, one to other fibronectin molecules, and another to specific cell surface receptors, so fibronectin can bind the ECM together as well as cells to the ECM. It is synthesised by a number of cells, including fibroblasts and endothelial cells (Jaffe and Mosher, 1978). Fibronectin is produced by blood vessels in response to injury (Clark et al 1982), when it aids in the repair process. Cells can attach to the matrix

through their interaction with fibronectin and this facilitates cell migration, which is an important event in embryogenesis, wound repair, and angiogenesis. For example, antibodies against fibronectin inhibit gastrulation in amphibian embryos (Boucaut et al,1984a).

Laminin

Laminin is a large (approximately 850 kD) complex of proteins arranged in the shape of a cross. There are multiple functional domains, including those that bind to type IV collagen, heparin sulphate, and specific cell surface receptors. Laminin is an ubiquitous component of basement membranes, and is the first ECM protein produced during embryogenesis (Timpl, 1989). It possesses the ability to link to cells and to other ECM components, and so stabilises the structures adjacent to basement membranes.

Vitronectin

Vitronectin was previously known as serum spreading factor and S-protein. It is present in the serum where it associates with platelets, and is found in the ECM (Hayman et al, 1983). Vitronectin has similar functions as fibronectin, including a role in phagocytosis, coagulation, and regulation of the complement system. It interacts with cells via specific cell surface receptors.

There are a number of other ECM molecules, including elastin, fibrinogen, von Willebrand factor, thrombospondin, entactin, tenascin, osteonectin, and osteopontin. Most of these interact with the same cellular receptors as fibronectin, collagen, laminin, and vitronectin, but they will not be described in more detail as they have not been a direct part of the work described in this section.

Basement Membranes

Basement membranes, or basal laminae, are specialized sheets of ECM that underlie all epithelial sheets and tubes, surround individual muscle cells, fat cells, and Schwann cells, and function as selective filters in the kidney glomerulus and lung alveolus. They form a barrier separating the cells from the connective tissue, although it can be traversed by neutrophils, monocytes, and lymphocytes. Following injury to tissue, the more resilient basement membranes form a scaffolding upon which new cells can migrate and attach, guiding appropriate regeneration.

The basement membrane supporting the endothelium is intimately involved in a number of important homeostatic processes. One of these is the movement of leucocytes from blood to the extravascular space, particularly during inflammatory responses, which will be discussed in more detail in section 4.

Another process is the adherence of platelets to the subendothelium following damage to the endothelium. The normal endothelium is non-adherent for platelets, but platelets possess receptors for a number of ECM molecules, including the collagen and laminin in the basement membrane (Hemler, 1990). They rapidly adhere to the exposed subendothelium and are then activated, which initiates haemostasis and thrombosis. Platelet ECM receptors will be discussed later in this section. Fibronectin is produced by the endothelial cells in response to the injury, and this forms a pathway to guide the proliferating endothelial cells to then cover the defect.

The basement membrane and ECM surrounding vessels influence the extravasation of blood-borne tumour metastases. Metastatic tumour cells adhere preferentially to the ECM underlying endothelial cells (Kramer et al, 1980), and laminin appears to be one of the important basement membrane component in this process (Liotta, 1986). The adhesion is mediated by specific cell surface receptors (Graf et al, 1987a). A number of tumour cells secrete matrix-degrading collagenases which aid the extravasation process (Liotta, 1986). Tumour cells which preferentially metastasise to a given organ adhere better to endothelial cells cultured on ECM taken from that organ (Pauli and Lee, 1988), suggesting that the endothelial cells express specific receptors that have been regulated by the underlying matrix. The role of inducible endothelial cell molecules in tumour invasion will be discussed in more detail in section 4.

Angiogenesis is another process where the endothelial cell basement membranes are intimately involved. This can occur in normal situations (eg inflammation, wound repair, delayed hypersensitivity reactions) and in pathological circumstances (eg tumours, chronic inflammatory responses, keloid formation). Angiogenesis can be studied in vitro using cloned endothelial cells, as they retain all of the information required to form three-dimensional tubular networks given the correct extracellular clues (Ingber et al Cell 1989). ECM molecules promote in vitro capillary organisation by dictating whether the endothelial cells proliferate, involute, or differentiate in response to soluble cues (Ingber and Folkman, 1989a, 1989b). Endothelial cells grown in culture without growth factor organise into capillary-like tubular structures

after several weeks (Folkman and Handenschild, 1980, Maciag et al, 1982). This occurs within days when cultured in three dimensional collagen gels (Montesano et al, 1983), and is stimulated by TGF-β (Madri et al, 1988). It occurs within hours when they are cultured in a reconstituted gel composed of basement membrane proteins (Matrigel, Collaborative Research Inc., Lultham, MA) (Kubota et al, 1988, Grant et al, 1989). Laminin is the principal factor in the Matrigel that induces this rapid differentiation, and it is mediated by two separate laminin domains that bind to endothelial cell receptors (Grant et al, 1989). The collagen type IV in the Matrigel makes a lesser contribution. The capillary-like structures in collagen-only gels are surrounded by a basement membrane including laminin secreted by the endothelial cells. The time taken to form this structure may explain the slower differentiation in these gels. These studies conclude that ECM components, principally laminin, are important controls during angiogenesis. The endothelial cells bind to the ECM components via specific cell surface receptors, which in turn interact with the cytoskeleton. This is a critical step, as capillary formation requires reorganisation of the cytoskeleton.

Receptor recognition of ECM proteins - the "RGD" sequence

ECM proteins frequently depend on the tripeptide sequence arginine-glycine-aspartic acid (Arg-Gly-Asp, RGD) for binding to their cell surface receptors (Ruoslahti and Pierschbacher, 1986). The attachment site in fibronectin has been narrowed down to a tetrapeptide (Arg-Gly-Asp-Serine) (Pierschbacher and Ruoslahti, 1984), in which the first three residues are essential for activity but the serine can be replaced by other amino acids. Synthetic peptides containing the RGD sequence can mediate cell attachment directly when bound to an insoluble substrate, or can inhibit cell attachment to fibronectin and other ECM proteins when in solution (Hayman et al, 1985).

Fibrinogen contains two RGD sequences and von Willebrand factor has one. RGD-containing peptides inhibit the binding of each of these proteins to platelets (Gartner and Bennett, 1985, Plow et al, 1985b). The same peptides also inhibit platelet aggregation induced by ADP, collagen, and thrombin, and platelet adhesion to fibronectin (Haverstick et al, 1985). The adhesion of fibronectin, fibrinogen, von Willebrand factor, and vitronectin to activated platelets is now known to be mediated by a single receptor, platelet glycoprotein IIb/IIIa (see later) (Pytela et al, 1986).

Vitronectin also has an RGD sequence that promotes cell attachment (Suzuki et al, 1985). The adhesion of vitronectin, von Willebrand factor, and fibrinogen to endothelial cells is inhibited by RGD-containing peptides, and is also dependent on a single receptor (vitronectin receptor - see later) (Cheresh, 1987d, Dejana et al, 1989). Endothelial cells express a distinct RGD-dependent receptor for fibronectin (Dejana et al, 1988b), and can also attach to the RGD sequence in the A chain of laminin (Grant et al, 1989).

Monocytes express an RGD-dependent receptor for fibronectin alone (Brown and Goodwin, 1988), and share with neutrophils another RGD-dependent receptor for multiple ligands that resembles the platelet GP IIb/IIIa and vitronectin receptors (Brown and Goodwin, 1988, Gresham et al, 1989). Antibodies against this receptor inhibit IgG-mediated phagocytosis that is stimulated by fibronectin, fibrinogen, vitronectin, von Willebrand factor, and collagen type IV.

Collagen type I has six separate RGD sequences, and a cell surface receptor for collagen that recognises this sequence has been identified (Dedhar et al, 1987a). This is probably a low affinity receptor and is distinct from other known collagen receptors.

The receptor for the complement protein fragment C3bi (complement receptor type 3, CR3) recognises a region that contains the RGD sequence (Wright et al, 1987), although RGD-containing peptides do not inhibit binding.

Tenascin, an ECM protein expressed during embryonic development and in some undifferentiated tumours, also binds to an RGD-dependent receptor (Bourdon et al, 1989).

Some cells will migrate in response to a fibronectin gradient, and this is inhibited by RGD-containing peptides (Straus et al, 1989). This again suggests that the cellular migration along fibronectin pathways during events such as embryogenesis and wound repair is dependent on RGD recognition by the cell surface receptors. Tumour cell lines selected for resistance to detachment by RGD-containing peptides specifically overproduce receptors for ECM proteins (Dedhar et al, 1987b, Dedhar et al, 1989). Penetration through human amniotic basement membrane and its underlying stroma by two melanoma cell lines and a glioblastoma cell line is inhibited by RGD-containing peptides (Gehlsen et al, 1988b). Finally, a novel protein engineered by introducing the RGDS tetrapeptide into a truncated form of protein A, is able to mediate cell attachment to a substrate (Maeda et al, 1989).

A number of these receptors have been purified and identified using affinity chromatography with RGD-containing peptides bound to sepharose, as well as the specific ECM proteins bound to sepharose.

The RGD mechanism of cell attachment is highly preserved through evolution. The RGD sequence is found in cell attachment proteins of other species, including a surface protein of Escherichia coli, a Sindbis virus coat protein (Pierschbacher and Ruoslahti, 1984), and rat bone sialoprotein (osteopontin) (Oldberg et al, 1986). The cell substrate attachment (CSAT) antigen is a receptor on chicken cells that has multiple ligands, including fibronectin, laminin, and possibly vitronectin and type IV collagen. These interactions can be inhibited by RGD-containing peptides (Horwitz et al, 1985, Menko and Boettiger, 1987). The linkage between plant cell wall and plasma membrane is mediated by an RGD-dependent recognition system (Schindler et al, 1989). RGD-containing peptides inhibit attachment to fibronectin and consequent cell invasion by the protozoan parasite Trypanosoma cruzi, which causes Chagas' disease (Onaissi et al, 1986). The aggregation protein discoidin I in the cellular slime mould Dictyostelium discoideum depends on an RGD sequence for its cellular recognition (Springer et al, 1984). Gastrulation in Drosophila embryos is prevented by the injection of RGD-containing peptides into the ventral periplasm (Naidet et al, 1987). Gastrulation in amphibian embryos is prevented by antibodies to fibronectin and RGD-containing peptides (Boucaut et al, 1984a, Boucaut et al, 1984b). The same peptides inhibit neural crest migration in avian embryos (Boucaut et al, 1984b). Lung metastasis by melanoma cells in a murine model is inhibited by an RGD-containing peptide (Humphries et al, 1986).

Thus, a great diversity of organisms has retained the RGD sequence as a recognition signal. The importance of this mechanism is also shown by the high degree of preservation through evolution of both the ECM proteins and their cell surface receptors.

Although the RGD sequence is important in cellular recognition of ECM substrates, it does not appear to account for the specificity of cell surface receptors for distinct ligands. Over 120 proteins are known which contain the RGD sequence, but many of these have no adhesive function. One suggestion is that RGD sequence recognition by different receptors is dependent on the surrounding sequences in the ligand (Ruoslahti and Pierschbacher, 1986). There is evidence that the RGD sequence serves as a shared binding site with a second binding site specific for each protein. A non-RGD-containing

sequence in the carboxyl-terminal segment of the γ-chain of fibrinogen also interacts with the platelet fibrinogen receptor GPIIb/IIIa (Kloczewiak et al, 1984). Two major adhesive recognition sites, which function synergistically, have been identified in fibronectin using site-directed mutagenesis (Obara et al, 1988). One contains an RGD sequence, but the other does not, and deletion of either results in significant loss of adhesive activity. The binding of endothelial cells and their differentiation into capillary-like tubes when grown on Matrigel is dependent on both an RGD-containing sequence in the A chain of laminin, and a YIGSR site in the B1 chain (Grant et al, 1989). A specific 67kD cell surface receptor recognises this YIGSR sequence (Graf et al, 1987a, Graf et al, 1987b), and this is a higher affinity interaction than that mediated by the RGD-dependent receptor. It has been postulated that this interaction creates the stable contacts for long-term attachment, whereas the RGD-dependent receptor is important for initial cell adhesion and for the transient contacts of motile cells (Basson et al, 1990). Synthesised YIGSR pentapeptide inhibits lung metastasis in mice injected with melanoma cells and inhibits invasiveness of the cells in vitro (Iwamoto et al, 1987), presumably by blocking tumour cell adhesion to the basement membrane.

Extracellular matrix receptors

Many of the cell surface receptors for ECM glycoproteins, and particularly those that recognise the RGD sequence, belong to a family of homologous molecules known as the integrins (Hynes, 1987). These receptors are all heterodimers consisting of an α chain non-covalently bound to a smaller β chain. The integrin family is further subdivided into subgroups on the basis of distinct β chains. Originally three groups (with β_1, β_2, and β_3 chains) were described, but more recently β_4 and at least two other β chains have been found. The different β chains are significantly homologous, as are the associated α chains, which suggests that they evolved from a single precursor receptor (probably for fibronectin) by a process of separate gene duplications.

Table 3 (page 103) summarises the integrin family.

This section first reviews the β_1 family, which in humans is often referred to as the very late antigens (VLA) family, followed by what is known about the β_4 chain. The introduction concludes with a review of the β_3 family, which includes the human cytoadhesins (platelet glycoprotein IIb/IIIa and the vitronectin receptor), and the current knowledge of other integrin β chains. The

β_2 integrins, which are not primarily involved in interactions with the ECM, will be discussed in section 4.

The β_1 integrin family

Cell surface receptors for ECM proteins have generally been defined by one of the following three experimental approaches:

(1) production of antibodies (monoclonal and polyclonal) against cell surface molecules, followed by the specific selection of antibodies that block cell adhesion to ECM components;

(2) affinity chromatography using ECM ligands or RGD-containing peptides bound to sepharose columns; and

(3) monoclonal antibodies produced against cell surface structures that were later shown to be ECM receptors.

Much of the current knowledge concerning this family of molecules stems from the original isolation of an ECM receptor on chicken cells using two monoclonal antibodies ("cell substratum attachment" [CSAT], and JG22) that inhibited cell adhesion to ECM components (Greve and Gottlieb, 1982, Neff et al, 1982). A similar receptor had been isolated from baby hamster kidney cells by a polyclonal antiserum (Wylie et al, 1979). These antibodies inhibit cell adhesion to vitronectin, fibronectin, laminin, and collagen types I and IV (Buck and Horwitz, 1987, Horwitz et al, 1985, Chen et al, 1985). The complex defined by these antibodies localises at sites of cell adhesion, and the purified antigen binds directly to ECM molecules (Buck and Horwitz, 1987). It is found in myogenic cells and fibroblasts in culture along stress fibres and at their termini in focal contacts with the substratum (Damsky et al, 1985). These sites correspond to extracellular fibronectin fibrils and intracellular cytoskeleton-associated molecules such as vinculin, talin, and α-actinin (Chen and Singer, 1982, Chen et al, 1985). These intracellular proteins are thought to form the linking chain between the cytoskeletal actin filaments and the cytoplasmic face of the plasma membrane, and are localised at sites of actin-membrane attachment. Talin is a major cytoplasmic protein implicated in the shape change that platelets undergo after activation (O'Halloran et al, 1985). Specific interaction between the CSAT complex and talin has been demonstrated (Horwitz et al, 1986) and is stimulated in lymphocytes by the phorbol ester phorbol 12-myristate 13-acetate (PMA) (Burn et al, 1988). Another protein has also been identified (fibulin) which is a potential mediator of interactions between the cytoskeleton and ECM receptors (Argraves et al, 1989). The

CSAT complex is persistently phosphorylated in chicken cells after transformation with the Rous sarcoma virus, which is initiated and maintained by the protein product of the viral v-src gene (Hirst et al, 1986). These cells exhibit decreased adhesiveness, loss of cell surface fibronectin, abnormal cell migration, dissolution of stress-fibre bundles, and ultimately a rounded, transformed cell morphology. It is probable that the phosphorylation interferes with the interaction between CSAT and the cytoskeleton. In contrast, differentiation of F-9 teratocarcinoma cells is associated with decreased phosphorylation of the complex which temporally coincides with the appearance of a highly structured actin cytoskeleton and accumulation of fibrillar fibronectin deposits (Dahl and Grabel, 1989).

These results demonstrate that the CSAT receptor complex functions as a receptor for ECM molecules and interacts with the cytoskeleton. The name integrin has been proposed for this complex to denote its role as an integral membrane complex involved in the transmembrane association between the ECM and the cytoskeleton (Tamkun et al, 1986).

The CSAT and JG22 antibodies both immunoprecipitate (by SDS-PAGE) a broad band of molecular weight 140kD under reducing conditions, and 3 bands of molecular weight 160kD (band 1), 135 kD (band 2), and 110kD (band 3) under non-reducing conditions (Knudsen et al, 1985). Further experiments showed that the complex is a mixture of 2 heterodimers in which band 3 is a common subunit bound to the distinct band 1 and band 2 subunits. Band 3 is now known as the integrin β_1 chain, and the other bands are α chains. CSAT and JG22 recognise epitopes on band 3, and so precipitate both dimers (Buck et al, 1986).

Molecules that are similar to CSAT and function as ECM receptors have been found in a number of other organisms. Monoclonal antibodies that inhibit the adhesion of fibroblastic Chinese hamster ovary cells to fibronectin bind to epitopes on the α and β chains of a 140kD (reduced) complex (Brown and Juliano, 1985, Brown and Juliano, 1988). The position specific (PS) antigens (PS1 and PS2) in Drosophila are found on cells of the dorsal and ventral compartments of the mature wing imaginal disc (Wilcox and Leptin, 1985). Each PS antigen is a heterodimer, and they share a common 110 kD chain that has 45% sequence identity to chicken and human β_1 subunits (MacKrell et al, 1988). Integrin α chains also have significant homology with the other PS antigens (Bogaert et al, 1987, Leptin et al, 1987), and so the PS antigens are members of the integrin family. They are necessary for wing development in Drosophila embryos (Brower and Jaffe, 1989), and appear to be involved in

muscle attachment (Bogaert et al, 1987). The lethal myospheroid mutation in Drosophila is due to an abnormality of the gene that encodes the PS antigen β subunit, and the embryos have defective musculature and a phenotype that suggests a defect of basement membranes (MacKrell et al, 1988). This indicates that the PS integrins are ECM receptors used to attach mesoderm to ectoderm during embryogenesis, and that they are required for the proper assembly of the ECM and for muscle attachment (Leptin et al, 1989). A role for integrins in muscle development in chickens has also been described (Menko and Boettiger, 1987).

ECM receptors that belong to the integrin family have also been found in the amphibian Xenopus laevis (DeSimone and Hynes, 1988), murine T lymphocytes (Maxfield et al, 1989), rat neurons and hepatocytes (Tomaselli et al, 1988, Gullberg et al, 1988), the nematode Caenorhabditis elegans, and the yeast Candida albicans (Marcantonio and Hynes, 1988). Antigens that mediate ECM adhesion but are less clearly related to the integrins have been described in sea urchins (Noll et al, 1985), staphylococcus aureus (Flock et al, 1987) and the cellular slime mould Dictyostelium discoideum (Gabius et al, 1985).

The integrin family has been highly preserved through evolution, which in itself shows the importance of ECM receptors. The family is highly developed in humans, and the remainder of this section discusses those receptors.

The human β_1 integrin (VLA) family

Human monocyte receptors for fibronectin were described in 1981 (Bevilacqua et al, 1981), and an anti-monocyte monoclonal antibody that blocked adhesion to fibronectin was produced by Hosein and Bianco (1985). In "Western" blotting experiments, it was shown to recognise a single chain of 110 kD (reduced and non-reduced). The clear demonstration of a human fibronectin receptor that belonged to the integrin family was provided by Pytela et al (1985a), who purified a 140 kD receptor from MG-63 human osteosarcoma cells on a fibronectin affinity column. The 140 kD protein was specifically eluted by RGD-containing peptides, and when it was incorporated into liposomes they bound to fibronectin (which was also blocked by RGD-containing peptides). MG-63 cells selected for resistance to detachment from fibronectin by RGD-containing peptides specifically overproduce this fibronectin receptor (Dedhar et al, 1987b). The amino acid sequence of the human fibronectin receptor has been determined (Argraves et al, 1987). The

α chain has 1008 amino acids and is 46% homologous to the α subunit of the vitronectin receptor (part of the cytoadhesion [β_3] family - see later). The β chain has 778 amino acids and is 85% homologous to the CSAT β chain (Tamkun et al, 1986), 44% to the human β_3 chain, and 47% to the human β_2 chain. These three β chains all have complete conservation of 56 cysteines in the extracellular portion, many of which are located in four repeating motifs within a cysteine-rich region in the carboxyl-terminal half of the molecule (Hemler, 1990).

The members of the human β_1 integrin family are often grouped under the title Very Late Antigens (VLA) of which the fibronectin receptor described above is VLA-5. The six VLA molecules are heterodimers with distinct α chains (designated α_1 to α_6) non-covalently linked to the human β_1 chain (Hemler, 1990) and they have all been implicated as cell surface receptors for ECM molecules. The β_1 chain has been designated CD29, and the α chains CDw49a-f. The name "VLA" comes from the original description of VLA-1 and VLA-2 as antigens that appeared on T lymphocytes after 2 to 4 weeks of in vitro stimulation, which is much later than other markers of T cell activation (eg IL-2 receptor, transferrin receptor, HLA class II) (Hemler et al, 1983). The monoclonal antibody that identified these antigens (A-1A5) binds to an epitope on the human β_1 chain. Five of the VLA family have been identified in studies with this antibody (VLA-1 to -5) (Hemler et al, 1987a) and their α subunit N-terminal amino acid sequences show an average of 42% homology to each other (Takada et al, 1987b). They also have 31 to 40% homology to α subunits from the human β_2 and β_3 families and the PS antigens in Drosophila. However, the short C-terminal cytoplasmic domains (15 to 53 amino acids) have very little homology to each other, which suggests that they may each have unique interactions with the cytoskeleton (Hemler, 1990). In contrast, the cytoplasmic domains of the β_1 subunits from different species are highly conserved.

The β_1 chain

The β_1 chain identified by A-1A5 has a molecular weight by SDS-PAGE of 130 kD under reducing conditions and 110kD under non-reducing conditions, which suggests that it has intrachain disulphide bonding (Hemler et al, 1987a). The gene for it has been mapped to chromosome 10 (Messer-Peters et al, 1984).

The β_1 chain is identical to platelet glycoprotein IIa (Pischel et al, 1988), and has been isolated from cultured human endothelial cells (van Mourik et al, 1985). VLA proteins have been found on almost all cell types examined (except red blood cells) and many cell lines (Hemler et al, 1987a, Hemler, 1988b). The monoclonal antibody A-1A5 does not bind to neutrophils (Hemler et al, 1987a), but another study using a different anti-β_1 monoclonal antibody isolated β_1 chains on neutrophils that associate with a possibly novel α chain to form a receptor for laminin (Bohnsack et al, 1990).

A number of monoclonal antibodies that recognise the human β_1 chain have been described in addition to A-1A5 (Hemler et al, 1984, Morimoto et al, 1985, Kandor et al, 1987, de Stooper et al, 1988, Akiyama et al, 1989a, Amiot et al, 1986, Brown et al, 1989). An interesting example is the monoclonal antibody 4B4, which was originally defined as a marker of the human T lymphocyte helper inducer subset that stimulates antibody production (Morimoto et al, 1985) and induces CD8-mediated cytotoxicity (Kalish et al, 1988). 4B4 was only later found to recognise the β_1 chain. Studies with 4B4 have shown that human memory T cells express high levels of CD29 along with a number of other adhesion related molecules (LFA-3, CD2, LFA-1, CD44 - see section 4) (Sanders et al, 1988a, Sanders et al, 1988b). Memory cells differ from naive cells in that they have been activated in the past (typically by antigen), and proliferate vigorously when restimulated with that antigen. Hence, CD29-high T lymphocytes are less common in the neonate (5% of the T lymphocytes in umbilical cord blood) than in adults (40% of T lymphocytes). CD29-high T lymphocytes also predominate in the lamina propria of the gut (James et al, 1986), synovial fluid of rheumatoid joints (Emery et al, 1987), and in perivascular locations in the skin (Bos et al, 1987), which are sites of particularly high exposure to antigen. It is possible that the higher expression of these adhesion molecules confers an increased adhesive capability that is important in the function of memory cells.

Little is known about the factors that regulate the cellular expression of the β_1 chain. Transforming growth factor-β_1 stimulates expression of the β_1 subunit and associated α subunits, as well as stimulating the production of ECM proteins (Heins et al, 1989, Ignotz and Massague, 1987).

VLA-1 ($\alpha_1\beta_1$, CD49a/CD29)

The original studies with the monoclonal antibody A-1A5 identified three distinct bands (molecular weights 210, 165, and 130 kD reduced) precipitated

from T lymphocytes that had been stimulated by phytohaemagglutinin (PHA) or by alloantigen (irradiated B lymphoblastoid cells) (Hemler et al, 1983). A single 130 kD band only was precipitated from resting T lymphocytes, but the other two bands appeared after several days of stimulation, particularly following the addition of exogenous IL-2. The β_1 chain migrates as part of the 130 kD band, and the 210 kD and 165 kD bands correspond to the α chains of VLA-1 and VLA-2 respectively. Another anti-β_1 antibody (TS2/16) was described in a later report (Hemler et al, 1984), as well as a monoclonal antibody (TS2/7) that was specific for the α chain of VLA-1 (Hemler et al, 1985b). Other anti-VLA-1 α chain monoclonal antibodies include IB3.1 (Bank et al, 1989), raised against a cloned IL-2-dependent cell line that expressed the $\gamma\delta$ T cell receptor, SR84 (Rettig et al, 1984), and S2G3 (Hall et al, 1990).

The molecular weight of VLA-1 under non-reducing conditions is 200 kD (α chain) and 110 kD (β chain), and the non-reduced molecular weight of the cross-linked dimer is 310 kD, showing that it exists as a 1:1 heterodimer (as do the other VLA molecules) (Hemler et al, 1987a).

The VLA-1 complex appears to define a novel late stage of T lymphocyte activation (Hemler et al, 1985a). It is found on nearly all long-term activated T lymphocytes (including those that have lost their IL-2 receptors), and on human T leukaemia virus-1 infected T lymphocytes (which have an activated T cell phenotype). It is co-expressed with VLA-2 on many but not all of these cells. VLA-1 is also expressed by activated thymocytes (Sanchez-Madrid et al, 1985). The relative expression of VLA-1 to other VLA molecules depends on cell growth conditions, and increases in cell cultures that are quiescent and deprived of serum (Fingerman and Hemler, 1988). Elevated VLA-1 is found on lymphocytes taken from the synovial fluid of patients with rheumatoid arthritis (Hemler et al, 1986) and in the peripheral blood of patients with multiple sclerosis (Hafler et al, 1985). It has been speculated that VLA-1 characterises a subpopulation of T cells that is activated but nonproliferating, and "compartmentalised" in certain parts of the body (Saltini et al, 1986).

VLA-1 is expressed in low amounts on peripheral blood monocytes but not on B lymphocytes or platelets (Hemler, 1990), and is also scarce on most cell lines (Hemler et al, 1984). It is found on a human neuronal cell line along with VLA-2 (Pischel et al, 1986), and on the T lymphoblastoid cell line C8215 and fibroblast line GM3349 (Pischel et al, 1987). In contrast, it is expressed in many tissues, particularly on the blood vessels (Hemler et al, 1984).

VLA-1 is a functionally important ECM receptor on certain cell lines. Neuroblastoma cell lines specifically selected for resistance to detachment

from purified fibronectin, collagen type I, and laminin by RGD-containing peptides, overproduce VLA-1 (Dedhar et al, 1989). The collagen receptors isolated by affinity chromatography from the human MeWo melanoma cell line are VLA-1 and VLA-2 (Kramer et al, 1989a). Neither are retained on fibronectin- or laminin-Sepharose columns. In contrast, a laminin receptor identified on neuronal cells is probably VLA-1 (Ignatius and Reichardt, 1988), and the anti-VLA-1 monoclonal antibody S2G3 inhibits the attachment of human JAR choriocarcinoma cells to collagen type IV and to the elastase fragment E1 of laminin (Hall et al, 1990). These differences in the ligands for the VLA-1 on various cell types suggests that there is some heterogeneity of the VLA-1 molecules expressed by these cells, or that other factors influence the selection of ligand.

VLA-2 ($\alpha_2\beta_1$, CD49b/CD29)

The VLA-2 molecule was identified on long-term activated T lymphocytes by the monoclonal antibody A-1A5 (Hemler et al, 1983). The molecular weight of the α chain is 165kD under reducing conditions and 150 kD non-reduced (Hemler et al, 1987a). The primary amino acid sequence of the α chain has 18 to 25% homology to other integrin α chains (Takada et al, 1989b).

Several monoclonal antibodies that bind to the α chain of VLA-2 have been described. 12F1 was used to demonstrate that VLA-2 is expressed by platelets, monocytes, lymphocytic cell lines, and fibroblasts (Pischel et al, 1987). The monoclonal antibody 5E8, which binds to many lung tumour cell lines and excised human lung tumours, is anti-VLA-2 (Zylstra et al, 1986). CLB-10G11 also recognises VLA-2, and was used to show that it is expressed by cultured HUVEC (Giltay et al, 1989). The ECM receptor for collagen (ECMRII), identified with the monoclonal antibody P1H5 (Wayner and Carter, 1987), is identical to VLA-2.

VLA-2 on platelets corresponds to the previously described platelet glycoprotein Ia/IIa complex (Pischel et al, 1988). Patients whose platelets lack glycoprotein Ia have defective collagen-induced platelet aggregation (Niewenhuis et al, 1985, Kehrel et al, 1988, Bienz et al, 1989). VLA-2 is the mediator of Mg^{++}-dependent adhesion of platelets to collagen (Santoro, 1986, Kunicki et al, 1988, Staatz et al, 1989), and it specifically binds to the $\alpha 1$(I)-CB3 fragment of collagen type I (Staatz et al, 1990). It is likely that it binds to an identical or similar sequence of amino acids in other types of collagen. Using the anti-VLA-2 monoclonal antibody GF1, Coller et al (1989)

demonstrated that collagen interacts directly with glycoprotein Ia/IIa suggesting that platelets can interact directly with exposed subendothelial collagen as well as indirectly via von Willebrand factor. However, VLA-2 does not mediate platelet adhesion to fibronectin or laminin (Sonnenberg et al, 1988b, Staatz et al, 1989).

The anti-ECMR II antibody P1H5 inhibits the adhesion of the human fibrosarcoma cell line HT-1080 to collagen types I and VI, but not to fibronectin or laminin (Wayner and Carter, 1987). VLA-2 is one of the collagen receptors (with VLA-1) that are expressed by the human MeWo melanoma cell line (Kramer et al, 1989a). Neither is retained by fibronectin- nor laminin-Sepharose columns.

In contrast, VLA-2 on cultured HUVEC is both a collagen and laminin receptor (Languino et al, 1989, and observations in this thesis), as well as on the human melanoma cell line LOX (Elices and Hemler, 1989). As there is no apparent structural diversity between the VLA-2 receptor from these different cell lines, it is unclear why VLA-2 on some cells is a collagen receptor alone, but on other cells is a collagen and laminin receptor.

VLA-3 ($\alpha_3\beta_1$, $CD_w49c/CD29$)

The α chain of VLA-3 has an apparent molecular weight of 150kD under non-reducing conditions, but when reduced separates into 135Kd and 25kD fragments which had been disulphide bonded to each other (Hemler et al, 1987a). The gene for the α chain is located on chromosome 17 (Rettig et al, 1984).

The VLA-3 heterodimer has immunological cross-reactivity with the chicken CSAT complex (Takada et al, 1987a), which has fibronectin and laminin receptor activity (Horwitz et al, 1985). VLA-3 is an ECM receptor with multiple ligands,like the CSAT complex, but may have lower affinity than other VLA receptors. It binds to immobilised laminin, but with less affinity than VLA-2 (Elices and Hemler, 1989), and to fibronectin and type IV collagen to some extent (Gehlsen et al, 1989). The ECM receptor ECMRI, recognised by the monoclonal antibody P1B5, is identical to VLA-3 (Takada et al, 1988). P1B5 inhibits cellular attachment to types I and VI collagen, and partially to fibronectin and laminin (Wayner and Carter, 1987). VLA-3 also binds to the protein invasin on the surface of the enteropathogenic bacterium Yersinia pseudotuberculosis, which mediates the entry of the bacterium into mammalian cells (Isberg and Leong, 1990). Invasin also binds to VLA-4, -5,

and -6, but not to other members of integrin families. Antibodies against the β_1 chain inhibit the invasin-promoted entry of bacterium into mammalian cells.

The VLA-3 α chain is also recognised by the monoclonal antibodies J143 (Fradet et al, 1984) and VM-2 (Kaufmann et al, 1989). It is expressed by most cell lines, particularly adherent ones, but is only weakly expressed or absent from blood cells and lymphoblastoid cells (Hemler et al, 1987a, Fradet et al, 1984, Hemler, 1990). VLA-3 is associated with the intercellular contact sites of cultured cells (Kaufmann et al, 1989), and its expression is increased by culturing adherent cells on ECM components (Kantor et al, 1987, Rettig et al, 1986).

VLA-3 is eluted from fibronectin affinity columns by salt rather than RGD-containing peptides (which elute the fibronectin receptor VLA-5 - see later) (Gehlsen et al, 1988a, Wayner and Carter, 1987), suggesting that VLA-3 is not an RGD-dependent receptor.

VLA-4 ($\alpha_4\beta_1$, CD49d/CD29)

The existence of a distinct VLA-4 structure was originally deduced from the study of cell lines that express VLA molecules but lacked the α chains of VLA-1,-2,-3, or -5 (Hemler et al, 1987a). This was proven when a specific anti-VLA-4 α chain monoclonal antibody (B-5G10) was produced (Hemler et al, 1987b). The VLA-4 α/β association is quite labile, and the α chain is susceptible to proteolysis. This explains why immunoprecipitates of VLA-4 often contain additional 80 and 70 kD proteins which are cleavage products of the α chain. The primary sequence of the α chain has a potential protease cleavage site near the middle of the coding region, which appears to explain the appearance of these fragments (Takada et al, 1989a). The sequence is 17 to 24% similar to other integrin α chains. The molecular weight of the α chain is 150kD under reducing conditions, and 140 kD when non-reduced. VLA-4 is identical to the lymphocyte surface antigen L25 (McIntyre et al, 1989), and antibodies against L25 inhibit cytotoxic T lymphocyte-mediated lysis of target cells, suggesting that VLA-4 has a role in cellular adhesion (Clayberger et al, 1987a). The monoclonal antibody HP2/1 also binds to the α chain of VLA-4 (Sanchez-Madrid et al, 1986).

VLA-4 is expressed on monocytes, lymphocytes, thymocytes, and most lymphoblast and myeloblast cell lines, but is weak or absent on most adherent cell lines (Hemler et al, 1987b). It is more prominent on cells that grow in

suspension, suggesting that its function may be different from the other VLA molecules.

VLA-4 appears to have a variety of functions. The anti-L25 monoclonal antibody induces antigen-independent homotypic aggregation of lymphocytes and human T and B cell tumour cell lines (Bednarczyk and McIntyre, 1990), which is inhibited by another anti-VLA-4 antibody B5G10. This aggregation is energy- and cation-dependent. An antibody (K20) binding to the β subunit of VLA-4 inhibits CD4-positive T lymphocyte proliferation triggered by CD2 or CD3, and the binding of K20 to activated T lymphocytes increases cyclic AMP levels (Groux et al, 1989). These experiments suggest that the VLA-4 molecule on T lymphocytes has a role in cellular activation and transduction of signals to the cytoplasm.

Other studies have shown that VLA-4 functions as a cell surface adhesion receptor. The α chain of VLA-4 is highly homologous to the α chain of the murine lymphocyte cell surface heterodimer LPAM-1 (lymphocyte Peyer's patch high endothelial venule (HEV) adhesion molecule-1 - see section 4) (Holzmann et al, 1989a), which mediates the adhesion of murine lymphocytes to HEV in Peyer's patch. The β chain of LPAM-1 is distinct from the integrin β_1 chain. Another important property of VLA-4 is that it binds to a newly described molecule on activated endothelial cells called vascular cell adhesion molecule-1 (VCAM-1) (Elices at al, 1990). This will be discussed in more detail in section 4.

VLA-4 also has ECM receptor function in that it binds to fibronectin, which has two distinct domains for cell attachment (Humphries et al, 1988). The first is located in the centre of the molecule (the central cell-binding domain), contains an RGD sequence, and is found in all fibronectin molecules. It is recognised by VLA-5 (or its equivalent) on a variety of cells. The second is in the type III connecting segment region of the molecule, which is alternatively spliced in a complex manner from precursor mRNA (Mould et al, 1990). It is a ligand for a more restricted group of cell types, and is not found in all fibronectin molecules. The most active cell binding site in this region comprises the first 25 amino acids, known as the connecting segment -1 (CS-1) site. VLA-4 is the lymphocyte and lymphocytic cell line receptor for this region (Wayner et al, 1989, Guan and Hynes, 1990, Mould et al, 1990, Garcia-Pardo et al, 1990), which has been previously defined as a fibronectin adhesion site for melanoma cells. Antibodies against VLA-4 partially inhibit lymphocyte adhesion to intact fibronectin, and specifically inhibit adhesion to fragments containing the CS-1 segment. This adhesion is not dependent on

RGD-recognition, and has been localised to a highly conserved 10 amino acid segment in the fibronectin. The binding epitope on VLA-4 for fibronectin is distinct from that for VCAM-1 (see section 4).

VLA-5 ($\alpha_5\beta_1$, CD_w49e/CD29)

The RGD-dependent human fibronectin receptor (Pytela et al, 1985a) is identical to VLA-5 (Hemler et al, 1987a, Takada et al, 1987a) and ECMR VI (Wayner et al, 1988). It has approximately 90% homology to the equivalent receptor in the mouse (Holers et al, 1989). The α chain has an apparent molecular weight of 150 kD non-reduced, but like VLA-3 (and VLA-6) separates into two fragments (135 and 25 kD) upon reduction. As a consequence, the α and β chains run as a broad band at about 130 kD under reducing conditions. The two fragments of the α chain are post-translationally processed from a single mRNA and linked to each other by disulphide bonds (Fitzgerald et al, 1987). The small fragment has a hydrophobic region near its carboxyl end that appears to be a transmembrane segment. The receptor can assemble and be inserted into the plasma membrane without complete oligosaccharide processing, but the oligosaccharide-mature form is necessary for its adhesion function (Akiyama et al, 1989b).

VLA-5 is expressed by lymphocytes and lymphocytic cell lines, monocytes, U937, platelets, fibroblasts, but is weak or absent on epithelial cell lines (Wayner et al, 1988, Garcia-Pardo et al, 1989, Brown et al, 1989). HUVEC express VLA-5 (Conforti et al, 1989, Languino et al, 1989), and when these cells are grown on fibronectin, the receptors cluster at focal contacts and are associated with intracellular vinculin and stress fibre formation (Dejana et al, 1988b). VLA-5 on platelets corresponds to part of the previously described platelet glycoprotein Ic/IIa (Pischel et al, 1988, Hemler et al, 1988a) (the other part is VLA-6 - see later), and functions as an activation-independent fibronectin receptor (Piotrowicz et al, 1988).

Antibodies against VLA-5 inhibit the binding of various cells to fibronectin, including lymphocytes, fibroblasts, platelets, endothelial cells, and VLA-5-expressing cell lines (Wayner et al, 1988, Charo et al, 1987, Akiyama et al, 1989a). They have no effect on adhesion to collagen or laminin. They also interfere with the assembly of an extracellular matrix by cultured cells by impairing the retention of fibronectin on the cell surface (Akiyama et al, 1989a). Some cell lines migrate on fibronectin, and this is specifically inhibited by anti-VLA-5 antibodies and by RGD-containing peptides (Straus et al, 1989).

Studies on Chinese hamster ovary cells which are motile on fibronectin suggest that their fibronectin receptors are recirculated back to the leading edge of the cell through the endocytic cycle (Bretscher, 1989), thus enabling the cell to attach itself to the substrate as it extends itself forward. Activated T lymphocytes express increased VLA-5 (Wayner et al, 1988) and adhere more strongly to the RGD-containing segment of fibronectin. This complements the VLA-4-mediated adhesion to the non-RGD site (Wayner et al, 1989). Treatment of the VLA-5-expressing erythroleukaemic cell line K562 with 12-O-tetradecanoylphorbol 13-acetate (TPA) reduces the binding of the cells to immobilised fibronectin, even though they express 10-fold more surface VLA-5 (Symington et al, 1989). The VLA-5 molecules expressed by these TPA-treated cells are functionally less efficient and have different electrophoretic mobility, due to altered N-linked glycosylation, possibly involving changes in sialylation.

VLA-6 ($\alpha_6\beta_1$, CD49f/CD29)

The α chain of VLA-6 was originally defined by the monoclonal antibody GoH3, which was developed as a marker of mouse mammary gland development (Sonnenberg et al, 1986). Since then two other antibodies have been reported (Hsi et al, 1987, Kennel et al, 1981, Hemler, 1990). Its molecular weight is 140 kD non-reduced and 120 kD reduced (plus a 30 kD chain). The amino acid sequence is 18 to 26% homologous to other integrin α chains (Tamura et al, 1990).

GoH3 recognises the human platelet Ic/IIa complex, and binds to epithelial cells in variety of tissues in both man and mouse, including the endothelium (Sonnenberg et al, 1987). The platelet Ic/IIa complex recognised by GoH3 is distinct from that defined by anti-VLA-5 monoclonal antibodies (Hemler et al, 1988a), although they are structurally very similar. The originally described platelet Ic is in fact composed of the α chains of VLA-5 and VLA-6, and hence platelets express VLA-2, -5, and -6 (Hemler et al, 1988a, Pischel et al, 1988). Platelets can adhere to laminin and fibronectin without being activated (Ill et al, 1984), and GoH3 inhibits platelet adhesion to laminin (Sonnenberg et al, 1988b). The major attachment domain in laminin for VLA-6 on human JAR choriocarcinoma cells is in the elastase fragment E8, which is distinct from the attachment site for VLA-1 (Hall et al, 1990, Sonnenberg et al, 1990). Therefore, the three VLA molecules on platelets mediate adhesion to collagen (VLA-2), fibronectin (VLA-5), and laminin (VLA-6).

The α chain of VLA-6 associates with the β_1 chain on other cell types, including monocytes, endothelial cells, fibroblasts, and some tumour cell lines (Hemler et al, 1989). However, it also associates with a different subunit to form a novel heterodimer on mouse mammary tumour cells (Sonnenberg et al, 1988a) and on a number of human epithelial and carcinoma cell lines (Hemler et al, 1989, Hsi et al, 1987). This subunit has been designated the integrin β_4 chain, as it has partial amino-terminal sequence homology to the other integrin β chains.

Other β_1 integrins

A 100 kD subunit has been described that associates with the β_1 chain but is electrophoretically and immunochemically distinct from the 6 VLA α chains (Kramer et al, 1989b). This complex was purified from human MeWo melanoma cells by affinity chromatography on laminin-sepharose columns (and not fibronectin- or collagen-sepharose columns), and so presumably functions as a laminin receptor on these cells.

Two antibodies against the β_1 chain precipitate a broad band at 125 to 135 kD (reduced and non-reduced) from neutrophils that is distinguishable from known VLA molecules (Bohnsack et al, 1990). The relationship between this complex and members of the VLA family is unknown.

The role of VLA molecules in cell signalling and T lymphocyte activation

There is now considerable evidence that VLA molecules have signal transducing functions. The make-up of the substratum influences the expression and deposition of ECM components by cells in culture, which suggests that the ECM receptors are transducing signals which alter the cellular phenotype (Streuli and Bissell, 1990). Similarly, mouse peritoneal macrophages are induced to accumulate GM-CSF mRNA and release GM-CSF by adherence to fibronectin as well as by inflammatory agents and phagocytosis (Thorens et al, 1987), and human monocytes are selectively induced to produce DNA encoding mediators of tissue inflammation and repair following adherence to ECM components (Sporn et al, 1990). Antibodies against the fibronectin receptor on rabbit synovial fibroblasts induce the expression of genes encoding the secreted ECM-degrading metalloproteinases collagenase and stromelysin (Werb et al, 1989),

demonstrating the transmission of signals via this receptor that enable the cells to influence their adjacent microenvironment. This induction of proteinases is inhibitable by the anti-inflammatory corticoid dexamethasone.

T lymphocytes adhere to immobilised fibronectin via VLA-4 and -5, and to immobilised laminin via VLA-6 (Kurki et al, 1987, Shimizu et al, 1990a). This binding is augmented upon cell activation without changes in the level of expression of the VLA molecules, which suggests that there is an alteration in the molecules themselves (structural, conformational, clustering) or in their adjacent microenvironment that increases the affinity for their ligands. This is in contrast to the situation on most cells where the VLA molecules appear to be constitutively avid for their ligands. However, the differentiation of T lymphocytes into the memory phenotype is accompanied by a several-fold increase in the expression of these receptors and a consequent more efficient binding to fibronectin and laminin.

Fibronectin synergises with anti-CD3 antibody to stimulate CD4-positive T lymphocyte proliferation in a serum-free culture system, and this is blocked by anti-β_1 antibody binding to the VLA-5 on the CD4-positive cells (Matsuyama et al, 1989). This suggests that increased expression of VLA-5 and its consequent adhesion to fibronectin are important contributions to the helper-inducer properties of CD29-high CD4-positive T lymphocytes. Other studies (Shimizu et al, 1990b, Davis et al, 1990) have found that both VLA-4 and -5 contribute to the fibronectin-facilitated proliferation, and also that laminin can facilitate CD3-mediated T lymphocyte proliferation (via the VLA-6 on the T lymphocytes). The VLA-5-mediated costimulation was blocked by RGD-containing peptides, whereas the VLA-4-mediated costimulation was inhibited by a 12 amino acid peptide derived from the alternatively spliced type III connecting segment of fibronectin. The costimulation of T lymphocyte proliferation by fibronectin and laminin was stronger than the costimulatory signals provided by cytokines such as IL-1β, IL-6, and IL-7. Another study has shown that immobilised collagen can also costimulate $CD4^+$ T lymphocytes with anti-CD3, via the VLA-3 complex and the CD26 molecule on the T lymphocytes (Dang et al, 1990).

These results suggest that the β_1 integrins can function as signalling molecules as well as adhesion molecules, and may influence antigen-specific T lymphocyte recognition. Thus they could serve the dual purpose of temporarily anchoring activated and/or memory T lymphocytes at sites of inflammation (eg during rejection) as well as facilitating the antigen recognition and activation that determines effector function.

The cytotoxic function of NK cells and lymphokine-activated killer (LAK) cells may also be dependent on these receptors, as antibody against laminin inhibits their cytolytic activity in a dose-dependent manner (Schwarz and Hiserodt, 1988).

The integrin β_4 chain

Two monoclonal antibodies that recognise the β_4 chain have been reported. S3-41 was produced against a human pancreatic cancer cell line, and identifies an antigen in a number of human carcinomas (Kajiji et al, 1987). The antigen is also present in a number of normal human tissues, particularly in the area of basement membranes, which is a very typical site for integrins. Another monoclonal antibody, 439-9B, identifies a complex on human and murine carcinomas which was named "tumour surface protein, molecular weight 180,000" (TSP-180) (Falcioni et al, 1988). TSP-180 is strongly expressed by highly malignant metastatic cells (Sacchi et al, 1989). The protein complexes recognised by these antibodies are identical and correspond to the β_4 chain that complexes to the α chain of VLA-6 (Kennel et al, 1989). So far, the β_4 chain has only been found in association with the α_6 chain.

The β_4 chain has a more complex precipitation pattern than is usual for integrin β chains, either because of differing contents of sialic acid (Kajiji et al, 1989) or due to proteolytic cleavage (Tamura et al, 1990). The three chains of β_4 (205, 180, and 150 kD reduced; 190, 160, and 130 kD non-reduced) run as a single chain of 100 kD (reduced) after the removal of sialic acid by neuraminidase. The deduced amino acid sequence of the β_4 chain has an extracellular portion homologous (35%) to other integrin β chains, but it has a unique cytoplasmic domain comprising more than 1000 residues (Tamura et al, 1990, Suzuki and Naitoh, 1990, Hogervorst et al, 1990). Studying the mRNA by the polymerase chain reaction suggests that multiple forms of the β_4 chain may exist.

The function of the β_4 complex is unknown, but it is probable that it is an epithelial cell receptor for ECM components, based on its tissue distribution and similarity to other members of the integrin family.

The β_3 integrin family

The other major family of human ECM receptors share the integrin β_3 chain, and are commonly called cytoadhesins (Plow et al, 1986, Ginsberg et al, 1988). The two well-defined members of this family are platelet glycoprotein IIb/IIIa and the vitronectin receptor. They share the β_3 chain (which is GP IIIa) non-covalently linked to distinct α chains. The β_3 chain is now designated CD61, platelet glycoprotein IIb as CD41, and the vitronectin receptor α chain as CD51.

Platelet glycoprotein IIb/IIIa (GP IIb/IIIa, $\alpha_{IIb}\beta_3$, CD41/CD61)

Patients with the bleeding disorder Glanzmann's thrombasthenia have platelets which fail to aggregate following agonist stimulation. The most common cause of this defect is the decreased platelet expression of the two proteins that make up the GP IIb/IIIa complex. Some patients have normal levels of GP IIb/IIIa, but there are abnormalities in the protein structure (reviewed: Phillips et al, 1988). The GP IIb/IIIa complex is the most abundant platelet cell surface protein, and constitutes 1 to 2% of the total platelet protein. The only other cells known to express it are megakaryocytes and the HEL human erythroleukaemia cell line (Zimrin et al, 1988), which has megakaryocyte-like properties. The IIb subunit consists of 125 kD and 25 kD chains linked by disulphide bonds (similar to the VLA-3, -5, and -6 α chains), and IIIa is a single 105 kD chain. The IIb subunit appears to run faster on gels under reducing conditions because of the dissociation of its two chains, and the IIIa subunit runs a little slower because of intrachain disulphide bonding. About 70% of GP IIb/IIIa is located on the platelet surface, and the remainder on cytoplasmic α-granules and cannaliculi connected to the surface. The intracellular association of the two subunits is necessary before either of them can be expressed on the cell surface (O'Toole et al, 1989).

The GP IIb/IIIa complex has no ligand-binding activity on unstimulated platelets, whereas after platelet activation (eg with thrombin), it binds fibrinogen, fibronectin, von Willebrand factor, and possibly vitronectin (Plow et al, 1984, Plow et al, 1985a, Pytela et al, 1986, Gardner and Hynes, 1985). The platelet activation appears to result in a structural or conformational change in the GP IIb/IIIa complex that activates its receptor function (O'Toole et al, 1990), and monoclonal antibodies have been described that selectively bind to GP IIb/IIIa on activated platelets (Shattil et al, 1985, Coller, 1985). Activation

allows larger molecules to bind to the receptor site than can bind to GPIIb/IIIa on unactivated platelets (Coller, 1986). Other changes that may occur upon platelet activation are alterations in the microenvironment around GP IIb/IIIa that may expose it to a greater extent, and clustering of the receptor (Phillips et al, 1988). The binding of ligands to IIb/IIIa also exposes new antigenic epitopes that may have roles in the events that follow, such as cell spreading, cytoskeletal rearrangements, and changes in gene expression (Frelinger et al, 1988), and monoclonal antibodies have been produced which preferential bind to these epitopes (Frelinger et al, 1990). Like other integrins, the ligand recognition requires the presence of divalent cations. One variant of Glanzmann's thrombasthenia has a point mutation in a region of the β_3 subunit that is highly conserved among integrin β subunits and is thought to be the cation-binding site. This mutation abolishes both cation binding and ligand recognition (Ginsberg et al, 1986, Loftus et al, 1990).

Active receptor sites for adhesion to subendothelium exposed by injury are present on unstimulated platelets. Collagen, fibronectin, and laminin bind to VLA-2, -5, and -6 respectively, and von Willebrand factor to glycoprotein Ib (Coller et al, 1983). The platelets are consequently activated, which is followed by aggregation, mediated by the GP IIb/IIIa. Fibrinogen binding to GPIIb/IIIa is necessary for platelet aggregation. The binding of fibronectin and von Willebrand factor probably have an additional role in aggregation as well as mediating further interaction with the subendothelium (Phillips et al, 1988). Platelet activation stimulates GP IIb/IIIa association with the cytoskeleton (Phillips et al, 1980, Painter et al, 1985), and contraction of the cytoskeletal filaments causes platelet shape change and clot retraction. The generation of the appropriate intracellular signals may depend on phosphorylation of tyrosine residues on platelet intracellular proteins, which is stimulated by ligands binding to GP IIb/IIIa (Ferrell and Martin, 1989).

GP IIb/IIIa possesses an RGD-dependent binding site. It is selectively bound to RGD-containing peptides immobilised on an affinity matrix, and the same peptides inhibit the binding of fibrinogen, fibronectin, and von Willebrand factor to GP IIb/IIIa on activated platelets (Pytela et al, 1986, Plow et al, 1985b, Haverstick et al, 1985, Gardner and Hynes, 1985). These peptides inhibit thrombin-induced platelet aggregation without altering the degree of platelet activation (Haverstick et al, 1985). Several venoms from pit vipers are GPIIb/IIIa antagonists and inhibit platelet aggregation (Dennis et al, 1989). They are homologous to each other, contain an RGD sequence, and bind reversibly with high affinity to both resting and ADP-activated human

platelets. One of them (kistrin) inhibits platelet aggregation in rabbits without inducing thrombocytopenia.

GPIIb/IIIa also has a non-RGD-dependent binding site. A synthetic 10 amino acid peptide from the extreme carboxyl terminus of the γ-chain of fibrinogen that does not contain an RGD sequence inhibits the binding of fibrinogen, fibronectin, and von Willebrand factor to GP IIb/IIIa on thrombin-stimulated platelets (including cells that are fixed after stimulation) (Plow et al, 1984, Kloczewiak et al, 1984).

Fibrinogen contains two RGD sequences, one near the carboxyl terminus of its α chain, and the other in the amino terminal region of the same chain. Platelet GP IIb/IIIa binds to the RGD sequence in the carboxyl terminal region as well as to the site at the carboxyl terminus of the γ-chain (amino acids 400-411) (Lam et al, 1987, Cheresh et al, 1989b). Peptides corresponding to either of these sequences inhibits the binding of GP IIb/IIIa to both sequences immobilised on columns (Lam et al, 1987). The amino terminal RGD sequence doesn't support cell attachment while still within the intact fibrinogen. It is probable that the IIb subunit interacts with the γ-chain sequence, and the IIIa subunit with the α chain RGD sequence (Santoro and Lawing, 1987). The RGD-binding site on IIIa has been localised to the region of amino acids 109 to 171 (D'Souza et al, 1988). This segment is highly conserved among the β subunits of integrins. A synthetic peptide corresponding to the terminal sequence of the γ-chain of fibrinogen cross-links to amino acids 294 to 314 on GP IIb (D'Souza et al, 1990, D'Souza et al, in press).

Monoclonal antibodies against platelet GP IIb/IIIa have been used to prevent reocclusion of coronary arteries after thrombolysis with tissue plasminogen activator (Yasuda et al, 1988). Platelet adhesion and aggregation at the site of damage are important contributors to reocclusion in this model. Anti-GP IIb/IIIa monoclonal antibodies also inhibit melanoma tumour growth (Boukerche et al, 1989), and the adhesion of tumour cells to platelets and tumour metastasis formation (Karpatkin et al, 1988). These investigations show that inhibiting platelet adhesiveness by blocking GP IIb/IIIa has promise for the treatment of clinical conditions.

Vitronectin receptor ($\alpha v\beta_3$, CD51/CD61)

The vitronectin receptor (VnR) shares the β_3 chain with platelet GP IIb/IIIa (Zimrin et al, 1988, Ginsberg et al, 1987), and has a distinct α chain which migrates at 150 kD under non-reducing conditions (Suzuki et al, 1986). The two chains that make up the α subunit are disulphide-linked, and the 25 kD chain contains the transmembrane segment (similar to GP IIb/IIIa). Platelet GP IIb and the α chain of the VnR show significantly homology to each other (Ginsberg et al, 1987).

VnR binds to RGD-containing sequences in its ligands (Pytela et al, PNAS 1985), which are vitronectin (Pytela et al, 1985b), fibrinogen, von Willebrand factor (Charo et al, 1987, Cheresh, 1987d), thrombospondin (Lawler and Hynes, 1989), and osteopontin (Reinholt et al, 1990). The α chain is involved in the RGD recognition (Cheresh and Spiro, 1987c). However, in contrast to GP IIb/IIIa, VnR does not bind to the non-RGD-containing terminal sequence of the γ-chain of fibrinogen (Smith et al, 1990b).

VnR is more widespread than platelet GP IIb/IIIa. It is expressed by platelets, where it is a receptor for vitronectin and thrombospondin (Lam et al, 1989, Lawler and Hynes, 1989, Thiagarajan and Kelly, 1988). Monoclonal antibodies against platelet GP IIIa also recognise a structure on cultured human endothelial cells (Thiagarajan et al, 1985, Leeksma et al, 1986, Dejana et al, 1988a) complexed to the VnR α chain rather than platelet GP IIb (Cheresh et al, PNAS 1987). Endothelial cells use VnR to adhere and spread on vitronectin, fibrinogen, and von Willebrand factor, and this is inhibited by anti-IIIa and anti-VnR antibodies and RGD-containing peptides (Charo et al, J.Biol.Chem.1987, Cheresh, 1987d, Preissner et al, 1988, Dejana et al, 1987 , Dejana et al, 1988a, Dejana et al, 1989). VnR cluster at sites of focal contacts and are associated with stress fibres when endothelial cells are cultured on these substrates (Dejana et al, 1987, Dejana et al, 1988b, Dejana et al, 1989). The spreading of endothelial cells on fibrinogen is slightly different from other ECM proteins because it requires endogenous fibronectin synthesis and secretion for proper organisation of the cytoskeleton (Dejana et al, 1990). Antibodies against the fibronectin receptor (VLA-5) and the VnR both inhibit the adhesion to fibrinogen, suggesting that adhesion to the secreted fibronectin matrix is necessary to maintain the attachment to fibrinogen.

Osteoclasts, which are bone marrow-derived cells important in bone resorption, express the VnR (Davies et al, 1989). It has been hypothesised that they use it to anchor to osteopontin bound to the mineral of the bone matrix at

sites of resorption (Reinholt et al, 1990). The synthesis of osteopontin is stimulated by calcitriol, which is known to induce bone resorption.

Macrophages (after a period of culture) (Krissansen et al, 1990), human smooth muscle cells, MG-63 fibroblast-like cells, and GM1380 fetal lung fibroblasts also express VnR (Plow et al, 1986, Charo et al, 1986).

A number of other cells express RGD-directed receptors that consist of the β_3 chain complexed to an α chain that is similar to the VnR α chain. These complexes may represent other members of the cytoadhesins family. M21 human melanoma cells adhere to vitronectin, fibronectin, and von Willebrand factor via a β_3 receptor that appears to be distinct from platelet GP IIb/IIIa and VnR (Cheresh and Spiro, 1987c). The diganglioside GD2 co-localises with this receptor on the cell surface and in focal adhesion plaques (Cheresh and Klier, 1986b, Cheresh et al, 1987b). Gangliosides are glycolipids that contain one or more sialic residues, giving them a net negative charge. Monoclonal antibodies against the gangliosides GD2 and GD3 inhibit the attachment of these melanoma cells (and neuroblastoma cells) to ECM proteins (Cheresh et al, 1986a). The gangliosides appear to synergise with the RGD-dependent receptor in cellular attachment to the ECM (Burns et al, 1988). Their role may be to produce a favourably charged microenvironment to facilitate peptide binding by the specific glycoprotein receptor. Oxidation of the terminal sialic acid residues by itself inhibits the cell attachment (Cheresh et al, 1986a).

Monocytes and neutrophils express a fibronectin receptor consisting of the β_3 chain and a distinct α chain (Brown and Goodwin, 1988). The myelomonocytic cell line U937 expresses a β_3 receptor after stimulation with 4β-phorbol 12-myristate 13-acetate (PMA) (Plow et al, 1986). The observation that IIb/IIIa complexes on neutrophils mediate their adhesion to fibronectin (Burns et al, 1986) has been attributed to "platelet dust" on the neutrophils (Ginsberg et al, 1988). The β_3-containing receptor on the neutrophils may provide an alternative explanation. Neutrophils also express a receptor that is distinct from other known integrins which binds to the RGD sequence in many ECM proteins (fibronectin, fibrinogen, vitronectin, von Willebrand factor, and collagen type IV) (Gresham et al, 1989). Monocytes and neutrophils utilise these VnR and VnR-like receptors for phagocytosis that is facilitated by the ECM proteins, particularly fibronectin and vitronectin. Recently, the macrophage VnR has been implicated in the phagocytosis of cells undergoing apoptosis (programmed cell death) (Savill et al, 1990), which is a process that is important in the physiological involution of organs, remodelling of embryonic tissues, and metamorphosis.

The VnR α chain is peculiarly promiscuous in that it associates with a number of different β chains. UCLAP3 lung adenocarcinoma cells express the VnR α subunit complexed with a novel β subunit (called $\alpha_v\beta_5$). This receptor is responsible for their RGD-dependent attachment to vitronectin and more weakly to fibronectin (but not to fibrinogen or von Willebrand factor) (Cheresh et al, 1989a). The same complex has also been identified in human placental extract (Smith et al, 1990a). The β_5 chain may be identical to the β_s subunit described by Freed et al (1989). The size and partial amino acid sequence of β_5 is very similar to β_1, β_2, and β_3 (Hemler, 1990), and so it has been designated a member of the integrin family. It is most homologous to the β_3 chain (56%) (McLean et al, 1990). The mRNA of the β_5 chain is found in more cell lines than β_3 mRNA, indicating that it may be widely distributed.

Cultured monocytes (macrophages) express both the typical VnR and a complex consisting of the VnR α chain and a β chain that is immunologically distinct from the β_3 chain (Krissansen et al, 1990). This "β_{3b}" subunit appears to be different from the β_5 chain, and its expression is regulated separately from the classical β_3 chain.

The VnR α chain also breaks one of the fundamental "rules" of the integrin family by complexing with the β_1 subunit on certain cell lines (Vogel et al, 1990, Bodary et al, 1990). On one cell type this complex is a fibronectin receptor, and a vitronectin receptor on another. It is the combination of chains in the receptor rather than the particular α chain that governs the ECM ligand.

The complexing of particular α chains with different β chains and the additional β chains that have been found means that the present categorisation of the integrins requires updating.

The integrin β_6 chain

A novel additional β chain has recently been identified using the technique of the polymerase chain reaction (Sheppard et al, 1990). The 788 amino acid sequence is 38 to 47% identical to the integrin β_1, β_2, and β_3 chains, and it has the 56 cysteine residues in the same positions in the extracellular domain. A unique feature is an additional 11 amino acids at the carboxyl terminus in the putative cytoplasmic domain, which suggests the possibility of distinctive interactions with cytoplasmic components. There is no published information as yet about the function of this β_6 chain or the α chains to which it is complexed.

TABLE 3: Integrin family of cell adhesion receptors.

Subunits	Names	Ligands	RGD role	Size (kD) Red.	Non-red
$\alpha_1\beta_1$	VLA-1, CD49a?/CD29	LM, CO	-	210/130	200/110
$\alpha_2\beta_1$	VLA-2, CD49b/CD29, gpIaIIa, ECMRII	LM, CO	-	165/130	160/110
$\alpha_3\beta_1$	VLA-3, CD49c?/CD29, ECMRI	FN,LM,CO	-	130+25/130	150/110
$\alpha_4\beta_1$	VLA-4, CD49d/CD29, LPAM-2	FN, VCAM-1	-	150/130	140/110
$\alpha_5\beta_1$	VLA-5, CD49e?/CD29, FNR, gpIcIIa, ECMRVI	FN	+	135+25/130	155/110
$\alpha_6\beta_1$	VLA-6, CD49f/CD29, gpIcIIa	LM	-	120+30/130	140/110
$\alpha_v\beta_1$	CD51/CD29	FN	+	125+24/130	150/110
$\alpha_L\beta_2$	CD11a/CD18, LFA-1	ICAM-1, ICAM-2	-	180/95	170/90
$\alpha_M\beta_2$	CD11b/CD18, Mac-1, CR3	C3bi, leishmania, LPS, factor X, FB	+?	170/95	165/90
$\alpha_X\beta_2$	CD11c/CD18, p150,95	?C3bi	?	150/95	145/90
$\alpha_{IIb}\beta_3$	CD41/CD61	FB, FN, vWF, VN	+	120+25/105	145/90
$\alpha_v\beta_3$	CD51/CD61, VNR	VN, FB, vWF, TSP	+	125+24/105	150/90
$\alpha_6\beta_4$	CD49f/CD?	?	-	120+30/220	140/210
				(180)	(165)
				(145)	(125)
$\alpha_v\beta_5$	CD51/CD?	VN, FN	+	125+24/110	150/100
$\alpha_4\beta_p$	CD49d/CD?, LPAM-1	?	?	150/?	140/100

Abbreviations: LM - laminin; CO - collagen; FN - fibronectin; FB - fibrinogen; vWF - von Willebrand Factor; VN - vitronectin; TSP - thrombospondin.

3.2: Production and characterisation of a monoclonal antibody (QE.2E5) which recognises the integrin β_1 chain

The monoclonal antibody QE.2E5 was produced in a fusion where the immunising cells were cultured HUVEC that had been stimulated with lipopolysaccharide (LPS) at a concentration of 1500 ng/ml for 4 hours. The culture supernatant was strongly positive when screened against cultured HUVEC by cellular ELISA, but there was no increased expression of the antigen on LPS-stimulated cells. The isotype of the antibody is IgG_{2b}.

Immunoprecipitation studies

The antigen recognised by QE.2E5 was first characterised by immunoprecipitation studies of lysates of ^{125}I-labelled cultured HUVEC, followed by analysis by one-dimensional SDS-PAGE. When run under reducing conditions, QE.2E5 immunoprecipitated a broad band at 130 to 160 kD on a 7.5% polyacrylamide gel. Under non-reducing conditions it immunoprecipitated two bands of molecular weights 150 and 120 kD. Similar complexes are found in studies using platelet lysates (see photograph page 134).

The molecular weights and changes in electrophoretic motility under reducing conditions of this complex of proteins are suggestive of members of the integrin family, in particular the VLA molecules (see the molecular weights in table 3, page 103). This hypothesis was tested by comparative immunoprecipitation studies performed with the anti-β_1 monoclonal antibody A-1A5 (Hemler et al, 1983) and the polyclonal anti-β_1 antiserum goat 172. QE.2E5, goat 172, and A-1A5 immunoprecipitate identical bands from cultured HUVEC (photograph page 134, lanes b, c, and d). QE.2E5 and A-1A5 also immunoprecipitate identical sized bands from platelets (photograph page 155, lanes c and d).

The VLA subunits all have isoelectric points between 4.8 and 6.2 (Hemler et al, 1988b), and have a characteristic immunoprecipitation pattern when separated by isoelectric focussing (IEF) followed by SDS-PAGE in the second dimension. In two-dimensional SDS-PAGE experiments, QE.2E5 and A-1A5 immunoprecipitate indistinguishable patterns from both HUVEC (photographs page 135) and platelet (not shown) lysates.

One dimensional immunoprecipitation studies were performed with QE.2E5 on a number of other cell types:

U937 (myelomonocytic cell line) - a broad single band is precipitated under reducing conditions, and two bands (a lower, broad band, and an upper, more defined band) without reduction (photograph page 136). This is consistent with the electrophoretic motility of VLA-5 (135/130 kD reduced, 150/110 kD non-reduced), which is the major VLA molecule expressed by U937 (Hemler et al, 1987a).

HL60 (myeloid cell line) - two broad bands appear under reducing conditions. The broad upper band migrates at 140 kD (photograph page 136), and covers the VLA heterodimers expressed by HL60 (Hemler et al, 1987b). Without reduction, this band separates into two bands (not shown). The lower band on reduced gels migrates at about 80 kD, which is consistent with the 80 kD band frequently observed when VLA-4 is immunoprecipitated. This has been characterised as the cleavage product of the α chain of VLA-4, which makes up a significant proportion of the VLA molecules expressed by HL60 cells (Hemler et al, 1987b).

K562 (erythroleukaemic cell line) - the weak bands immunoprecipitated by QE.2E5 (not shown) are similar to those on U937 and consistent with VLA-5, which is virtually the only VLA molecule expressed by K562 (Hemler et al, 1987a).

JM (T lymphocytic cell line) - broad band at 135 kD with reduction and two bands without reduction; a weak 150 kD band and a stronger 110 kD band (photograph page 143, lanes a and c). This will be discussed further in section 3.3.

Jurkat (T lymphocytic cell line) - weak bands only were precipitated from Jurkat cells (not shown). The dimeric pattern was more easily distinguishable on gels run without reduction.

All these patterns are consistent with the VLA molecules known to be expressed by these cell lines.

This evidence shows that QE.2E5 immunoprecipitates members of the β_1 integrin family, and suggests that the epitope is on the β chain. This is confirmed in Western blotting studies as the antibody recognises a 110 kD band from HUVEC and platelet lysates (without reduction) (photograph page 137), which is the expected position for the β_1 chain. The antibody does not blot under reducing conditions, suggesting that the epitope is destroyed by that treatment. An additional 90 kD band is blotted in the platelet lysates, which is consistent with the size of the intracellular precursor of the β chain (Jaspers et al, 1988).

Tissue distribution of the antigen recognised by QE.2E5

A variety of techniques (indirect immunoperoxidase staining, cellular ELISA, flow cytometry, immunoprecipitation) were used to examine the cellular and tissue distribution of the antigen recognised by QE.2E5.

(1) Cells

QE.2E5 recognises cell surface molecules expressed by the following cells and cell lines:

- cultured HUVEC (demonstrated by indirect immunoperoxidase [IP], cellular ELISA, flow cytometry, and immunoprecipitation)
- platelets (IP, immunoprecipitation)
- monocytes (IP, flow cytometry)
- T lymphocytes (IP, flow cytometry)
- U937, HL60, K652 (IP, immunoprecipitation, flow cytometry)
- Jurkat (IP, immunoprecipitation)
- JM (immunoprecipitation, flow cytometry)

The anti-β_1 monoclonal antibody A-1A5 also binds to these cell types. In addition, QE.2E5 binds to mononuclear cells purified from the peripheral blood of rabbits, pigs, and sheep (demonstrated by flow cytometry).

The expression of the QE.2E5 antigen (and the β_1 chain recognised by A-1A5) on human neutrophils was examined by flow cytometric analysis (see page 138), in view of the disagreement about the presence of β_1 chains on these cells (Hemler, 1990). Both antibodies weakly but clearly stain neutrophils in unseparated whole blood. Neutrophils were then purified from peripheral blood and stained (flow cytometry) with QE.2E5 and A-1A5, plus a panel of monoclonal antibodies against the individual VLA α chains. Both QE.2E5 and A-1A5 clearly stain the purified neutrophils, and the staining patterns obtained with the antibodies directed against the individual VLA α chains suggest that VLA-5 and VLA-6 are the principal molecules that they express.

(2) Tissues

The distribution of the QE.2E5 antigen was examined in a number of tissues by indirect immunoperoxidase. The patterns of staining in the skin, spleen, lymph node, salivary gland, and kidney were directly compared with A-1A5 and found to be identical. The distribution of QE.2E5 alone was assessed in tonsil, skeletal muscle, and peripheral nerve.

Skin - staining is confined to the basal cells of the epidermal layer, adjacent to the epidermal-dermal junction (photographs page 139).

Spleen - the predominant staining is in the red pulp and the trabeculae. The histiocytes and macrophages in the cords, and the endothelial cells lining the sinusoids, are positive. The trabeculae and fibrous capsule stain in a fibrillar pattern, corresponding to the long cytoplasmic processes of the connective tissue fibroblasts. The intima of blood vessels and cells within the media of large vessels are positive. The white pulp is notably negative apart from infrequent blood vessels and occasional branching cells that may be fibroblasts or dendritic cells. The lymphocytes appear to be negative (photographs page 140).

Lymph node - the fibrous capsule and bands stain strongly in a fibrillar pattern, and the intima and media of blood vessels are positive. The only stained cells in the body of the node are infrequent fibroblasts and macrophages. Lymphocytes are negative (photograph page 141).

Salivary gland - the only positive areas are the endothelium of blood vessels and the base of the acini, in the regions of the contractile myofibrils. The ducts are negative.

Kidney - QE.2E5 stains the glomerular and intertubular capillaries, the intima and media of larger vessels, mesangial cells, parietal epithelial cells lining Bowman's capsule, the fibrous capsule, and the basal portions of tubular cells adjacent to their basement membranes. The cytoplasm of the tubules is negative (photograph page 141).

Tonsil - the pattern of staining is similar to the spleen and lymph node, with the fibrous capsule, blood vessels, and interstitial fibroblasts all positive. The only positive cells in the follicles are the occasional macrophages and fibroblasts. The other cells within the follicles and the rim of mantle lymphocytes are all negative.

Skeletal muscle - the capillaries between the individual muscle fibres, the intima and media of the larger vessels, and the outer borders of each muscle

fibre are all strongly positive. The perimysium that surrounds each muscle fasciculus is positive in a fibrillar pattern (photograph page 142).

Peripheral nerve - the capillaries, intima and media of larger vessels, perineurium, and endoneurium are all strongly positive. Other connective tissue is moderately positive in a fibrillar pattern (photograph page 142).

Functional studies with QE.2E5

The functional effects of QE.2E5 was examined in several assays where β_1 integrins are known to or expected to have functional significance.

(1) Cellular adhesion to ECM components

Cells can adhere to purified ECM components if they express the appropriate receptors, and this is inhibited by polyclonal antiserum against the β_1 chain (Takada et al, 1987a). Studies to be described later in this section demonstrated that cultured HUVEC adhere and spread on purified fibronectin, collagen types I and IV, and laminin by means of the VLA molecules that they express on their surface. In contrast, the cell lines U937 and K562, which only express the fibronectin receptor VLA-5 in significant amounts, adhere well to fibronectin but not to collagen or laminin.

Cell-matrix adhesion assays were performed to examine the effects of QE.2E5 on these interactions, but no inhibition was observed.

(2) Mixed lymphocyte culture (MLC)

The composition of the substrate has important influences on cellular proliferation, and the activating signals can be transmitted to the cells via their extracellular matrix receptors (discussed in the literature review at the commencement of this section). Therefore, it is possible that the β_1 integrins have a role in the proliferation of allogeneic mononuclear cells in the MLC, and that blocking these receptors might have some inhibitory effect. However, QE.2E5 had no effect on the MLC-induced proliferation between several pairs of HLA-non-identical donors.

(3) Mixed lymphocyte endothelial reaction (MLER)

For similar reasons, it is possible that the β_1 integrins are important signalling molecules in the initiation of proliferation of mononuclear cells co-cultured with allogeneic HUVEC, and that inhibiting the interaction between these receptors and their ligands might diminish the proliferative response. However, QE.2E5 had no consistent effect on cell proliferation in a number of MLER experiments performed using multiple mononuclear cell donors and different HUVEC cell lines.

(4) Cellular adhesion to cultured HUVEC

Low numbers of the T lymphocyte cell line Jurkat adhere to unstimulated cultured HUVEC, but more adhere to LPS-stimulated HUVEC. As will be discussed in section 4, this increase in adhesion is largely mediated by the interaction between VLA-4 on the Jurkat cells and an inducible molecule on the HUVEC (VCAM-1). QE.2E5 binds to the VLA-4 molecules expressed by the Jurkat cells, but has no effect on their adhesion to either unstimulated or LPS-stimulated HUVEC.

(5) Platelet aggregation

Platelets can be induced to aggregate by a number of agents, including ADP, collagen, ristocetin, arachidonic acid, adrenaline, and thrombin. The chief mechanism of this aggregation is the interaction between activated platelet GPIIb/IIIa and fibrinogen. However, the initial adhesion of collagen to platelets is via GP Ia/IIa (VLA-2).

The effect of QE.2E5 on stimulated platelet aggregation was kindly performed by the Coagulation Laboratory in the Department of Haematology at The Queen Elizabeth Hospital. The anti-GPIIb/IIIa monoclonal antibody 25E11 (Burns et al, 1986) inhibits both ADP- and collagen-induced aggregation. However, QE.2E5 does not inhibit platelet aggregation induced by either agent. The lack of effect on collagen-induced aggregation suggests that QE.2E5 does not significantly interfere with the initial adhesion of collagen to the VLA-2 expressed by the platelets.

Discussion

These studies show that QE.2E5 recognises the same molecule as the anti-β_1 monoclonal antibody A-1A5. The other heterodimeric antigen complexes expressed by cultured HUVEC are the vitronectin receptor and MHC molecules, but their reported molecular weights are distinct from those of the complexes immunoprecipitated by QE.2E5.

QE.2E5 immunoprecipitates the same bands as A-1A5 and the anti-β_1 antiserum, both by one-dimensional SDS-PAGE and in two-dimensional gels after isoelectric focussing in the first dimension. The patterns of precipitation show that QE.2E5 binds to the β_1 chain rather than any of the α chains. In addition, it recognises a band at 110 kD (non-reduced) in Western blotting, which is the predicted weight of the β_1 chain under those conditions. The extra band blotted at 90 kD from platelet lysates must possess the same epitope as the larger chain, and is probably the intracellular precursor of the β_1 chain that has been identified in other studies (Hemler et al, 1987a, Jaspers et al, 1988). As this precursor is an intracellular protein, it is not available for cell surface radiolabelling with ^{125}I by the lactoperoxidase method used in this thesis, and was not seen in the immunoprecipitation studies. The studies of Jaspers et al showed that the β_1 chain is synthesised in the endoplasmic reticulum as a precursor with a polypeptide core of 73kD and high-mannose oligosaccharide side chains. The mature cell surface form is derived by conversion of the precursor to a larger endoglycosidase H (endo H)-resistant glycoprotein that contains fucose.

The cellular distribution of the antigen recognised by QE.2E5 and its identity to that of A-1A5 is further evidence that QE.2E5 binds to the β_1 chain. The binding of QE.2E5 to mononuclear cells from other species is not surprising considering that the β_1 sequence is highly preserved along the evolutionary chain. The only other molecules known to be expressed by endothelial cells, platelets, and monocytes are the vitronectin receptor (VnR) and HLA class I molecules. The complex identified by QE.2E5 has molecular weights and changes in electrophoretic motility under reducing conditions that clearly distinguish it from both of these molecules.

One confusing observation is that both QE.2E5 and A-1A5 stain neutrophils by flow cytometric analysis. This is contrary to the original observations with A-1A5, but in agreement with another study using different antibodies where β_1 integrins were found to be laminin receptors on neutrophils (Bohnsack et al, 1990). The staining presented here suggests that

the β_1 chains are mainly present as VLA-5 and VLA-6, which could function as fibronectin and laminin receptors respectively on the neutrophils. However, another study (Wayner et al, Nov.1988) found that neutrophils failed to stain with a different antibody against VLA-5, but were weakly stained by an anti-VLA-2 antibody. Some of these disagreements could be due to differences in techniques used in these studies, but the question of neutrophil expression of β_1 integrins remains to be resolved. It could be studied by performing parallel experiments at the same time with all of the different anti-β_1 antibodies that are available.

The staining with QE.2E5 in all tissues examined exhibits a consistent pattern. It is found where cells have direct contact with basement membranes (eg basal cells of epidermis, around skeletal muscle cells, the base of tubular cells, adjacent to Bowman's capsule in the glomerulus), on endothelial cells (which are adherent to the supporting ECM in the wall of the vessel or around the vessel, and which also can interact with circulating fibronectin), on macrophages, and on the fibroblasts that manufacture most of the ECM itself. In these positions, all of these cells are directly interacting with ECM components. The basal cells of the epidermis are a very characteristic site for integrin receptors, as these cells are adjacent to the basement membrane that separates the epidermis from the dermal connective tissue. It is likely that these receptors are important in maintaining integrity of the dermal-epidermal interface. A-1A5 stains the tissues with an identical pattern, which is further evidence that QE.2E5 recognises the β_1 chain.

QE.2E5 has no inhibitory effect in a variety of assays where cellular adherence to the ECM is or probably is of importance. Parallel studies with A-1A5 showed that it does not inhibit cellular adhesion to purified ECM components, which suggests that it also does not bind to a functionally important epitope on the β_1 chain. Previous studies had already shown that a polyclonal anti-β_1 antiserum inhibits cellular adhesion to the ECM (Takada et al, 1987), which implies that the β_1 chain alone has some adhesive function for ECM molecules. However, most of the specific recognition function of the ECM receptors resides with the α chain, as will be discussed later. It appears that the epitopes recognised by QE.2E5 and A-1A5 on the β_1 chain have no direct role in ECM adhesion.

The evidence shows that the monoclonal antibody QE.2E5 recognises an epitope on the integrin β_1 chain, which adds to the small list of published anti-β_1 antibodies (see introduction).

3.3: QE.2E5 and A-1A5 define different but overlapping subgroups of β_1 chains

A series of sequential immunoprecipitation studies with QE.2E5, A-1A5, and the polyclonal anti-β_1 goat antiserum were performed to attempt to further demonstrate that QE.2E5 recognises an epitope on the integrin β_1 chain. In these studies, the process of mixing the cell lysate with one of the antibodies, followed by removal of the antigen/antibody complexes with staphylococcus aureus cells, is repeated until the lysate is completely depleted of the antigen recognised by that antibody. This usually requires 4 to 5 cycles of immunoprecipitation. The lysate is then immunoprecipitated with the other antibody of interest, and the antigens obtained in each of the steps analysed by SDS-PAGE. If two antibodies recognise the same cell surface antigen, there will be no antigen remaining in the lane corresponding to the last immunoprecipitation step. Conversely, if the antigens differ, a detectable band will be present in the last lane despite all the preclearing steps.

The cells used for these studies were HUVEC, platelets and the JM T lymphoid cell line. They had all been surface labelled with ^{125}I.

On two separate occasions each with HUVEC and platelets, the sequential immunoprecipitation studies with QE.2E5 and A-1A5 gave the same result (further studies were not possible due to insufficient A-1A5 antibody). After completely preclearing of the lysate of the complexes recognised by QE.2E5, a small but significant amount of heterodimer was still precipitated by A-1A5. The two bands were in the same position as those precipitated normally by A-1A5 and QE.2E5. Following complete preclearing of a separate lysate of the complex recognised by A-1A5, QE.2E5 precipitated a small but significant amount of antigen, but on this occasion it ran as a single band at the same molecular weight as the β_1 chain (photograph page 143).

These experiments suggest that there is heterogeneity of the β_1 chains, and so further studies were performed with the polyclonal anti-β_1 antiserum. This antiserum was produced by injecting goats with purified β_1 antigen and will recognise multiple epitopes, and so should bind to any possible alternative β_1 chains.

Following complete preclearing of a lysate of HUVEC of the antigen recognised by QE.2E5, the polyclonal antiserum precipitates a small amount of heterodimer (photograph page 144). This is identical to the pattern observed when precipitating the QE.2E5-cleared lysate with A-1A5. After preclearing a separate HUVEC lysate with A-1A5, the polyclonal antiserum

precipitates mainly a single band at a molecular weight corresponding to the β_1 chain, plus a barely detectable band at the level of the α chains (photograph page 144). This pattern is almost identical to that observed after QE.2E5 precipitation of the A-1A5 precleared lysate, with the exception of the weak higher molecular weight band. Attempts to preclear a HUVEC lysate completely with the polyclonal antiserum were unsuccessful. If that manoeuvre were successful, it is expected that it would completely remove all the complexes recognised by both QE.2E5 and A-1A5.

Flow cytometric analysis of JM T lymphoid cells showed that they express significant quantities of both VLA-4 and VLA-5 (not shown). This is consistent with the immunoprecipitation pattern obtained with A-1A5, namely, a broad band at 130 to 145 kD with reduction, and 110 kD and weak 150 kD bands without reduction. However, unlike HUVEC and platelets, the bands precipitated with QE.2E5 in the same experiment had slightly different molecular weights. Under reducing conditions, the broad band precipitated by QE.2E5 had a slightly higher molecular weight, and under non-reducing conditions, the β chain precipitated by QE.2E5 had a slightly lower molecular weight. The weaker α chain bands ran at the same molecular weight for both antibodies (photograph page 143).

A sequential immunoprecipitation study was attempted with JM cells, but was not completely satisfactory for two reasons. The preclearing step could only be performed with QE.2E5 because of insufficient A-1A5 antibody, and the cells labelled quite poorly with the ^{125}I. Nevertheless, after complete preclearing of the lysate by QE.2E5, it appeared that a weak single band (reduced) was still precipitated by the A-1A5 (not shown).

Discussion

The studies with HUVEC and platelets demonstrate that there are at least three subgroups of the integrin β_1 chain. The epitopes recognised by QE.2E5 and A-1A5 are both present on most β_1 chains, as each antibody preclears a majority of the chains precipitated by the other. A small subgroup possesses the QE.2E5 epitope only, and a third has the A-1A5 epitope only. The chains that have the A-1A5 epitope alone are precipitated as intact heterodimers, whereas those with the QE.2E5 epitope alone precipitate as a single band. This appears to represent β chain without associated α chain, as the VLA α and β chains have clearly different molecular weights under the non-reducing conditions in these studies, and should migrate as clearly distinguishable

separate bands. There is evidence that the integrins can only be expressed on the cell surface as intact α-β complexes (O'Toole et al, 1989), and it is possible that these β_1 chains were originally complexed to α_4 or α_6 chains, as VLA-4 and VLA-6 readily dissociate during immunoprecipitation studies (Hemler et al, 1987b, Hemler et al, 1988a).

The sequential immunoprecipitation study with JM cells, while technically not entirely satisfactory, supports this finding on HUVEC and platelets. However, a more significant finding with JM cells is the difference in the molecular weights of the bands precipitated by QE.2E5 and A-1A5. This alone suggests that the two antibodies are recognising distinct (although overlapping) subgroups of the β_1 chain.

These findings demonstrate that there is heterogeneity of the β_1 chains expressed by these cells. This is not unique, as heterogeneity of a number of cell surface molecules, including members of the VLA family, has been described. This can be manifested as polymorphism of an antigen between different individuals, or as different forms of the antigen expressed by different cell types.

An example of polymorphism of a member of the VLA family is the Br^a/Br^b alloantigen system on platelets, which has been localised to the GP Ia/IIa complex (VLA-2) (Kiefel et al, 1989, Woods et al, 1989). Other integrin polymorphisms are the Zw^a alloantigen system, which is located on the β_3 chain (Giltay et al, 1988), and that identified by the monoclonal antibody RM2.184, which identifies a polymorphic determinant on the complement receptor for C3bi (CR3), a member of the β_2 integrin family (Russ et al, 1985) (see section 4). The basis of the latter polymorphism is unclear, as there is no variation in molecular weight or electrophoretic mobility, but the epitope is probably on the α chain. CD44 is another adhesion molecule which will be discussed in the next section, and it bears the polymorphic blood group antigen In(Lu) (Picker et al, 1989). In addition, the two different Pgp-1 allotypes in mice is most likely due to a single amino acid change in the CD44 molecule between different strains (Nottenburg et al, 1989).

There are also a number of examples of heterogeneity of cell surface molecules between different cell types, including with members of the β_1 integrin family. The β_1 subunits expressed by the U937 and HL60 cell lines are larger than those on K562 cells (Hemler et al, 1983). This is attributed to variable N-linked glycosylation, because after enzymatic removal of N-linked carbohydrate, the β subunits all migrate at the same molecular weight (Hemler et al, 1985b). Stimulation of K562 cells with phorbol esters increases their

surface expression of fibronectin receptors, but alters their electrophoretic mobility (again due to changes in N-linked glycosylation). These altered fibronectin receptors have a significantly decreased affinity for fibronectin (Symington et al, 1989). Similarly, the fibronectin receptors on T lymphocytes and fibroblasts share immunologic determinants, but the molecular weight of the lymphocyte protein is larger (Cardarelli and Pierschbacher, 1987). Two different cDNAs for β_1 subunits have been found in the amphibian Xenopus laevis (DeSimone and Hynes, 1988). They have 98% identity in amino acid sequence, and are 82% identical to the human β_1 sequence.

The structural relationship of the alternative β_3 chain (β_{3b}) (Burns et al, 1990) to the classical β_3 chain is unknown, but they are distinguishable by standard physicochemical methods. The β_{3b} chain migrates faster on SDS-PAGE after reduction, and has a distinct isoelectric point and a different one-dimensional peptide map. A cDNA encoding an alternative cytoplasmic domain for the β_3 subunit has also been described (van Kuppevelt et al, 1989).

There are additional examples of heterogeneity within the β_2 integrin family. The monoclonal antibody S6F1 identifies heterogeneity of the LFA-1 molecule (Morimoto et al, 1987), and the α and β subunits of LFA-1 have multiple glycosylated isoforms, largely as a result of different degrees of sialization (Pardi et al, 1989). The expression of these isoforms varies between different lymphocyte subsets. The shared β_2 chains of LFA-1 and Mac-1 are glycosylated differently, even though they are simultaneously synthesised in the same cells (Dahms and Hart, 1986). The α and β subunits are assembled before the Golgi-mediated oligosaccharide processing, demonstrating that the quaternary structure can influence the site specific glycosylation of the protein.

The Hermes lymphocyte homing receptor (CD44), which participates in the adhesion of lymphocytes to high endothelial venules in peripheral lymph nodes, mucosal lymphoid tissue, and the synovium of inflamed joints (see the next section), is also heterogeneous. Polyclonal antiserum against CD44 inhibits lymphocyte adhesion at all these sites, whereas monoclonal antibodies have been produced that selectively inhibit adhesion at different sites (Jalkanen et al, 1987). CD44 has variable protein structure (Kansas et al, 1989) and glycosylation (Jalkanen et al, 1988, Pals et al, 1989b), which differs between cell types, and the encoding mRNA also varies according to the cell type (Stamenkovic et al, 1989).

Heterogeneity is not restricted to adhesion molecules. A determinant which is found only on the HLA-DR molecules expressed by lymphocytes and

not by monocytes or their precursors, is recognised by a particular monoclonal antibody (Torok-Storb et al, 1983). In one patient with melanoma, the HLA-DR molecules on their melanoma cells and on their Epstein-Barr virus transformed B lymphoblastoid cells had different molecular weights, due to glycosylation variations resulting from differences in asparagine-linked oligosaccharide processing (Alexander et al, 1984).

All of these molecules are involved in cellular recognition functions, and have detectable heterogeneity, which is due to changes in amino acid sequence or number, and/or different post-translational modifications. The studies in this thesis show evidence for heterogeneity of the integrin β_1 chain. However, the subgroups that express only one of the two epitopes represent a small percentage of the total number of VLA molecules. They are not distinguishable on HUVEC or platelets by molecular weight or isoelectric point, which suggests that they either represent amino acid changes that do not significantly alter the weight of the polypeptide backbone, or glycosylation differences that do not affect the migration during electrophoresis. However, the heterogeneity on JM cells is sufficient to produce a detectable alteration in the molecular weights of the chains. Unfortunately, attempts to determine if this was due to variations in N-linked glycosylation by treating the cells with tunicamycin did not give clearcut results.

There are a number of possible reasons for cells expressing different forms of the β_1 chain. The integrin β chains may mediate transmembrane signalling following the binding to ligand (van Noesel et al, 1988), and the association of an α chain with different β chains could generate multiple signals in response to a single matrix protein. The α chains are generally thought to control the fine specificity of the integrin receptors, although the association of α_v with a different β chain (β_5) results in different ligand specificity (Cheresh et al, 1989a). The ligand specificity of some VLA molecules also varies between cell types. VLA-1 is a collagen receptor on melanoma cells (Kramer et al, 1989a) but a laminin receptor on neuronal cells (Ignatius and Reichardt, 1988). VLA-2 is a collagen receptor alone on platelets (Staatz et al, 1989, Kunicki et al, 1987), the melanoma cell line MeWo (Kramer et al, 1989a), and fibroblasts (Elices and Hemler, 1989), whereas it is a collagen and laminin receptor on cultured HUVEC (Languino et al, 1989) and the melanoma cell line LOX (Elices and Hemler, 1989). The VLA-2 molecules from the different cell types are physically and immunochemically indistinguishable. One possibility is that subtle variations in the β chains between cell types mediates the different ligand specificities. The observation

of different subgroups defined by QE.2E5 and A-1A5 is evidence for this hypothesis, but it is difficult to test further as neither antibody has functional effects that can be examined on different cell types.

3.4: Studies with antibodies against specific VLA α chains

HUVEC express members of the β_1 integrin family, and the following studies were designed to investigate their function on endothelial cells and relevance to alloimmunity. A panel of specific monoclonal antibodies directed against the β_1 chain and each of the VLA α chains was assembled and used to study specific aspects of each of the VLA molecules. The sources of the antibodies and their relevant characteristics are described in the Materials and Methods section.

3.4 (1) Expression of VLA molecules by cultured HUVEC

Two methods were employed to study the expression of VLA molecules on cultured HUVEC - cellular ELISA and flow cytometric analysis. There was good concurrence between the results obtained with both methods, and the HUVEC expressed each of the VLA molecules to some extent (assessed as weak, moderate, or strong positive). The flow cytometric analysis is shown on page 145.

Strongly positive - β_1, VLA-2
Moderately positive - VLA-3, VLA-5
Weakly positive - VLA-1, VLA-4, VLA-6

Discussion

Cultured HUVEC express varying amounts of all six VLA molecules as well as a variety of other adhesion molecules (see literature review for this section and section 4). This wide reportoire is not surprising, as they adhere readily to a variety of substrates, and have multiple complex interactions with leucocytes (see section 4).

The finding of VLA-1, -2, -3, -5, and -6 on the HUVEC is not unexpected, as the cells can attach to collagen, fibronectin, and laminin, which are the ligands for these receptors. As will become clear in subsequent studies of the tissue expression of the VLA molecules, the HUVEC expression of these particular receptors is in good agreement with their distribution on vascular endothelium in tissue sections. However, it is surprising to find VLA-4 on the HUVEC, as this molecule has been confined to non-adherent cells in other studies (Hemler, 1990). The VLA-4 expression was weak but consistent on

multiple batches of HUVEC, and found using both assays. There are two possible explanations for this finding. One is that it is a correct observation, and that VLA-4 is present in low amounts on HUVEC, where it has an uncertain function. The other explanation is that it is an artifact, either because of the presence of small amounts of contaminating leucocytes (unlikely, because even multiply passaged HUVEC that had been growing for several weeks were weakly positive), or due to nonspecific binding of the particular anti-VLA-4 antibody used. The latter possibility could be examined by comparison with other antibodies against VLA-4, but these were not available during the course of these experiments. It is important to note that the isotype matched negative control antibody was consistently negative during these studies.

Studies have documented the presence of the β_1 chain and VLA-2 on cultured HUVEC (van Mourik et al, 1985, Giltay et al, 1989). The results presented here are generally in agreement with the only published comprehensive examination of HUVEC expression of VLA molecules (Languino et al, 1989). However, this study did not find significant expression of VLA-4 using a different antibody against that molecule, which further suggests that the finding of VLA-4 is dependent on the antibody used. Otherwise, the level of expression of the β_1 chain and the other 5 VLA molecules was of the same order as that described here. Another study has also identified VLA-2, VLA-3, and VLA-5 as important receptors for a variety of substrates on cultured endothelial cells from several sources (Albeda et al, 1989).

3.4 (2) Expression of VLA molecules in the normal human kidney

The distribution of the VLA α chains and β chain in the human kidney was examined using the indirect immunoperoxidase technique described in Materials and Methods. Sections had been cut from PLP-fixed biopsies taken from normal kidneys just prior to their use as transplants. Sections were also stained with the anti-β_4 antibodies S3-41 and 439-9B.

Results:

β_1 chains - widely distributed in the human kidney. They are present on the glomerular and intertubular capillaries, intima and media of larger vessels, mesangial cells, parietal epithelial cells lining Bowman's

capsule, fibroblasts in the intertubular areas, and at the base of all tubules, adjacent to their basement membranes. The tubular cytoplasm and visceral epithelial cells in the glomerular tuft are negative (photograph page 146).

VLA-1 - virtually identical to the distribution of the β_1 chains, apart from a slight decrease in mesangial staining.

VLA-2 - weak and variable glomerular staining, not clearly restricted to either the mesangial or endothelial cells. The intertubular areas and intertubular capillaries are negative, but there is weak staining of the intima of larger vessels. The proximal tubules are all negative, whereas the basal membranes of distal tubular and collecting duct cells are positive, and this staining often spreads to the lateral surface between adjacent cells without reaching their apex (photograph page 146).

VLA-3 - the distribution on tubular cells is the same as that for VLA-2, and it is also strongly expressed on the glomerular capillaries and the parietal epithelial cells which line Bowman's capsule. The mesangial cells are negative. The intertubular areas are weakly stained, but the intertubular capillaries are negative. The intima of larger vessels is weakly positive (photograph page 147).

VLA-4 - no expression seen in the kidney.

VLA-5 - weak expression in the glomerulus (capillaries), and some weak intertubular capillary staining. The clearest staining is on the intima of large vessels, but only of moderate intensity (photograph page 147).

VLA-6 - the glomeruli, intertubular capillaries, and tubular cytoplasm are negative. The intima of large vessels is weakly positive. The basal membranes of all tubular cells are positive, and this staining is stronger at the base of the proximal tubules (photograph page 148).

β_4 chains - no expression seen in the kidney.

Discussion

The individual VLA molecules have distinct distributions in the normal human kidney. This presumably reflects the different requirements of particular cells for adhesion to their adjacent ECM, and may be partly dictated by the distribution of specific ECM components.

The distribution of the β_1 chains is in agreement with that found in other studies (Hemler et al, June 1984, de Strooper et al, 1989, Korhonen et al, 1990a). This last study examined the distribution of the β_1 (and β_3) chains in

human foetal and adult kidneys, and found the β_1 chains on the basal surfaces of glomerular endothelial cells, podocytes abutting the glomerular basement membrane, the basal aspect of proximal tubular epithelial cells, and more diffusely in the epithelial cells of the distal tubules and collecting ducts. A separate study localised the β_1 chains by immunoelectron microscopy to the cell membranes of mesangial, epithelial, and endothelial cells that face the mesangial matrix or the glomerular basement membrane (Kerjaschki et al, 1989).

The distribution of the individual VLA molecules is in good agreement with a recent study (Korhonen et al, 1990b), and corresponds closely to the distribution of laminin, collagen type IV, and fibronectin found in a study of the rat kidney, taking into account their individual ligand specificities (Courtoy et al, 1982). In this study, laminin and collagen type IV were mainly found in the basement membranes of glomerular and peritubular capillaries and tubular epithelium, Bowman's capsule, and the media of arterioles. In contrast, fibronectin was absent or weak in these sites, and mainly found in the mesangial matrix, endothelial-pericyte matrix, and on the intima of arterioles. All of the VLA molecules are found on endothelium to some extent in the kidney (except VLA-4), which is in good agreement with the expression of VLA molecules by cultured HUVEC.

The distribution of VLA-1 almost matches that of the β_1 chains, and its localisation suggests that it is an important participant in cellular adhesion to a variety of basement membranes. The expression on the intima of large vessels is consistent with its presence on cultured HUVEC (see above and Languino et al, 1989). The parietal epithelial cells of the glomeruli express VLA-1 as well as VLA-3, and these appear to be the β_1 integrins that mediate their attachment to the basement membrane in the formation of Bowman's capsule. VLA-1 functions as a collagen or laminin receptor on cell lines which have been studied in detail, and it is likely that it has a similar role in intact tissue. An earlier study with the anti-VLA-1 antibody TS2/7 found it to be less widespread in the kidney (Hemler et al, 1984). However, the sections used in that study were cut from frozen, unfixed tissue, whereas the PLP fixation used here gives better preservation of the fine detail of the kidney.

VLA-2 is weakly and inconsistently expressed in the glomerulus. However, it has a distinctive distribution at the base of distal tubules and collecting ducts but not proximal tubules. VLA-2 is a collagen receptor on a number of cell types (eg platelets) and also a laminin receptor on some (eg cultured HUVEC) (Elices and Hemler, 1989, Languino et al, 1989), and so it is

likely that it also is involved in cellular adhesion to the basement membranes at these sites in the kidney.

VLA-3 is expressed on the basal membranes of the same subgroup of tubules as VLA-2, and again presumably is a mediator of adhesion of these cells to that particular subgroup of basement membranes. The distal tubular and collecting duct cells express VLA-1, -2, -3, and -6, whereas the proximal tubules only express VLA-1 and -6. This implies that there are unique distinguishing features about the basement membranes of these subgroups of tubules. This may be relevant to the differentiation and functions of the tubular cells which these basement membranes support. A clinical situation where this could be important is when the kidney is damaged by a process that causes acute tubular necrosis (eg ischaemia). Provided that the degree of damage does not preclude functional recovery, the more resilient basement membranes will survive the insult relatively untouched and maintain the correct "pattern" for appropriate regrowth of the tubules. It is possible that the heterogeneity of the basement membranes "programmes" the tubules to regrow in their previous form and with the same functional characteristics. The variation in tubular cell receptors for the basement membrane laminin and collagen is evidence for this heterogeneity, and the different integrin receptors may transmit ECM-dependent messages that govern the resulting tubular phenotype. Irreversible cortical necrosis can follow more severe ischaemia, and the consequent disordered fibrosis rather than orderly regrowth of tubules may be secondary to critical damage to the tubular basement membranes.

VLA-3 is also strongly expressed on glomerular capillaries and may have an important role in glomerular integrity and interaction with soluble fibronectin in the plasma and fibronectin attached to the endothelial cells. An immunofluorescence study with the same anti-VLA-3 antibody as used here also documented the prominent glomerular expression (Cordon-Cardo et al, 1984), but they found no tubular staining. This disagreement may be due to different sensitivity of the two staining techniques.

VLA-4 is not expressed in the human kidney. This is not surprising as it is generally only found on circulating haematopoietic cells (Hemler, 1990), but is inconsistent with the earlier finding that cultured HUVEC express some VLA-4 (see above). As discussed before, this could be due to an artifact of the staining of the HUVEC, or represent a situation where HUVEC expression of an antigen does not equate with its expression on vascular endothelium in intact tissue.

VLA-5 is a fibronectin receptor only and has a restricted distribution in the kidney which matches the distribution of fibronectin. The glomerular expression is weak and much less intense than that of the other fibronectin receptor VLA-3. VLA-5 is mainly found on the intima of large vessels where it presumably interacts with plasma fibronectin and fibronectin attached to the endothelium, rather than with that which is in the extravascular space. Plasma fibronectin is a soluble dimer which is involved in coagulation, wound healing, and phagocytosis through opsonisation (Mosher, 1984), and fibronectin is produced in response to injury by blood vessels, when it acts as a crucial guide during the regrowth of damaged endothelium (Clark et al, 1982). The endothelial VLA-5 may have a crucial role in these homeostatic processes.

In addition to weak staining of the intima of large vessels, VLA-6 is found on the basal portions of tubular cells, particularly on the proximal tubules. VLA-6 is a laminin receptor (Sonnenberg et al, 1988b, Shimizu et al, 1990a, Hall et al, 1990), and may therefore contribute to the adhesion of tubules to the laminin in the basement membrane. It is usually found in tissue sections at the interface between epithelial cells and their basement membranes (Sonnenberg et al, 1987). The VLA-6 α chain is only complexed with the β_1 chain in the human kidney as the β_4 chain is not expressed in any of the sections.

The prominent expression of the β_1 integrins in the human kidney suggests that they are important for the maintenance of long-term tissue stability. In a solid organ such as the kidney, the ECM (and particularly the basement membranes) forms the supporting scaffold to maintain tissue integrity. The cells need therefore to form firm attachments to the ECM. The integrin ECM receptors are important for the specific recognition of particular ECM proteins, and they mediate the initial attachment of cultured cells to purified ECM components. In vivo studies have shown that integrins also mediate the transient adhesion of migrating cells to the ECM (Straus et al, 1989).

There is in vitro evidence that non-integrin adhesion receptors may be particularly important for the long-term stabilisation of cell-substrate attachments, and it is probable that stable cell-substrate attachments are maintained in vivo by a combination of these receptors and the integrin ECM receptors. The 69 kD protein LB69 is a cell surface receptor for laminin that is not a member of the integrin family (Yannariello-Brown et al, 1988), and is only organised on the surface of cultured cells after cell-substrate attachment is well established (several hours) (Basson et al, 1990). It attaches to a

pentapeptide sequence tyrosine-isoleucine-glycine-serine-arginine (YIGSR) that is unique to the B1 chain of laminin (Graf et al, 1987a). This is a higher affinity interaction than that mediated by integrin receptors for laminin, and LB69 may be an important mediator of stable cell attachment to the laminin in basement membranes in vivo.

In addition to the fibrous proteins, the proteoglycans in the ECM interact with cells and with other ECM proteins. Some proteoglycans are also attached to the surface of cells and can bind to collagen and fibronectin to mediate cell adhesion (Ruoslahti, 1989). This appears to be another auxilliary mechanism that complements and stabilises the more specific integrin-mediated adhesion.

Another possible mechanism of cell attachment to the ECM may be via cell surface gangliosides. These are glycolipids that contain one or more sialic residues, which gives them a net negative charge. They may contribute to attachment to the ECM by producing a favourably charged electrostatic microenvironment that facilitates ligand binding by the specific receptor (Burns et al, 1988).

An alternative and complementary role of the integrin ECM receptors is the transmission of ECM-dependent signals to the interior of the cell. In vitro studies have shown that the interaction of ligands with integrins results in complex cellular responses including changes in cell shape (Hedin et al, 1988), cell proliferation (van Noesel et al, 1988), and gene expression (Thorens et al, 1987, Werb et al, 1989). It is possible that the β_1 integrins in the kidney convey signals from the ECM that influence the differentiation and function of the attached cells. This complements the tissue stabilising function of the integrin and non-integrin receptors.

3.4 (3) Expression of VLA molecules during renal allograft rejection

The same antibodies and staining technique were used to examine the expression of the VLA molecules in 8 renal allografts that had been biopsied during an episode of dysfunction. Six of these specimens had been frozen unfixed, and so the architecture was not quite as well preserved (particularly that of the glomeruli). In addition, infiltrating leucocytes and damage due to rejection tend to obscure the detail of the kidney.

Each of the specimens had been independently assessed by a histopathologist. Three were diagnosed as representing cellular and vascular

rejection, 4 as cellular rejection alone, and 1 as cyclosporine toxicity without any rejection.

Staining of the kidney substance

As far as could be determined, the pattern of distribution of each of the VLA molecules in the glomeruli and around the tubules was identical to the normal kidneys. The only change in expression was that of VLA-3 and VLA-5 on the larger blood vessels. This was most prominent in the cases of vascular rejection, where VLA-3 was significantly increased on the intima and walls of involved arterioles and arteries (photograph page 148), and VLA-5 was increased on the intima (photograph page 149). Changes in their expression in the cases of cellular rejection and cyclosporine toxicity were not convincing.

Staining of the infiltrating leucocytes

The leucocytes infiltrating a rejecting renal allograft are a heterogeneous population largely composed of macrophages, lymphocytes, and NK cells (see section 2.1). It was not possible in this study to reliably distinguish these cells, and so only general observations about their expression of VLA molecules are possible. Most collections of infiltrating cells in the rejecting grafts contained cells that were stained by the antibodies against the β_1 chain, but frequently more than half of the cells in a collection were negative. The cells consistently failed to stain with the antibodies against VLA-2, VLA-3, VLA-5, and VLA-6. The most frequently expressed molecule was VLA-1 (6 out of the 7 rejecting kidneys to varying degrees) (photograph page 149), and some VLA-4 positive cells were found in 4 out of the 7. In one particular example, most of the cells immediately adjacent to the lumen of a large vessel expressed VLA-4, whereas the cells further away were negative (photograph page 150).

In contrast, virtually all infiltrating cells consistently and strongly express HLA class I molecules.

Discussion

The exclusive increase in expression of the two fibronectin receptors VLA-3 and VLA-5 on larger blood vessels during rejection is probably related to the increased production of fibronectin by endothelium in response to inflammation (Clark et al, 1982). It may be that the increased availability of

ligand (fibronectin) stimulates the production of the appropriate receptor. A study of mouse macrophages has shown that inflammatory stimuli increase their production and expression of the murine equivalent of VLA-5 (Holers et al, 1989). The prominent expression of VLA-3 in the walls of the inflamed arteries may be due to greater expression on activated fibroblasts which are known to lay down the increased matrix characteristic of the fibrointimal proliferation that accompanies vascular rejection.

The pattern of expression of VLA molecules on the infiltrating leucocytes is unexpected as it is quite different to the pattern on circulating leucocytes (Hemler, 1990). Peripheral blood T lymphocytes and monocytes express significant amounts of VLA-5 and VLA-6 as well as VLA-4. Monocytes also express moderate levels of VLA-2. However, both cell types express very little VLA-1. In contrast, the leucocytes infiltrating the rejecting allografts apparently only express VLA-1 and VLA-4, and a number lack detectable VLA molecules altogether.

There are several possible explanations for these observations. The VLA-1 positive cells could represent an activated but nonproliferating population of "compartmentalised" cells similar to that described in rheumatoid synovial fluid and the lung (Hemler et al, 1986, Saltini et al, 1986, and discussed in the introduction to this chapter). Their presence in these rejecting kidneys suggests that they have an important role in the alloimmune response. It is not surprising that VLA-4 is present, but it is only found on a limited number of cells. The apparent decrease in expression with increasing distance from the blood vessel in one case suggests the possibility that there is active downregulation of the receptor once the cell has left the circulation. As will be discussed in section 4, a major role of VLA-4 could be in the adhesion of T lymphocytes to activated endothelium at sites of inflammation, and once this has occurred, the VLA-4 might have less importance and so be downregulated or shed. Similarly, the surprising lack of VLA-5 and VLA-6 in the infiltrating cells suggests that they have also been actively shed or downregulated once the cells have passed through the vessel's basement membrane. While laminin is essentially confined to the basement membrane, and therefore VLA-6, as a laminin receptor, may not have any useful role within the extravascular space, fibronectin is found outside the circulation, and it might be expected that the cells would retain VLA-5 for attachment to it in this compartment. Conceivably, VLA-1 and to a lesser extent VLA-4 could be the major ECM receptors for cellular attachment to and migration through the extravascular space at sites of inflammation such as allograft rejection.

There are some drawbacks to this method for assessing the expression of cell surface molecules in infiltrating cell populations, including the inability to simultaneously identify the precise cell type that is expressing a certain receptor. One possible method for making more accurate and sensitive judgements is using two-colour flow cytometric analysis of cells obtained by aspiration biopsy of the renal allografts (Totterman et al, 1989). However, this system for monitoring grafts is not being used at present in the renal transplantation unit at The Queen Elizabeth Hospital.

3.4 (4) Expression of VLA molecules in the spleen

The expression of VLA molecules plus the β_4 chain was studied on acetone-fixed sections of normal human spleen, with the same antibodies used on kidneys in section 3.4 (2).

The β_1 chain is very prevalent in the spleen (see section 3.2). It is found on the endothelial cells and walls of all vessels (including the sinusoids), on the splenic cords in the red pulp, and in the connective tissue trabeculae in a fibrillar pattern. The white pulp is negative apart from occasional small blood vessels and branching cells which may be fibroblasts. The lymphoid cells are negative in all areas.

VLA-1 is almost identical apart from its absence from the luminal surface of the endothelium of large vessels. However, it is clearly expressed at the interface between the endothelial cells and the media. The distribution of VLA-2 is also similar to the β_1 chains, although it is generally weaker in the red pulp and trabeculae, and it is particularly strong on the thin strip of non-lymphoid cells that surround the white pulp. VLA-3 is strongly expressed on the intima and media of blood vessels (apart from the sinusoids), and weakly on the trabecular connective tissue, but is not found elsewhere (photograph page 150). VLA-4 is only found on occasional interstitial cells which appear to be macrophages or dendritic cells. VLA-5 is confined to the intima of large vessels.

VLA-6 has a quite different distribution from the other VLA molecules. It is expressed by the endothelium of large vessels and the antibody also precisely picks out the endothelial cells that line the sinusoids, without staining the cells in the cords or the trabecular connective tissue (photograph page 151). Under high magnification, the sinusoidal staining is confined to the basal membrane of the endothelial cells, presumably adjacent to their basement membranes. The β_4 chain is strongly expressed in the spleen, in a very similar distribution

to VLA-6 (photograph page 151). This suggests that some (or all) of the α_6 chains are complexed to the β_4 subunit in the human spleen.

Discussion

The VLA family is widely distributed in the spleen, but there is significant variation between the individual molecules. The distribution of the individual VLA molecules is different from that of the ECM components that they bind to (eg laminin occurs in all basement membranes but the laminin receptors are more selectively expressed). This suggests that they are more than just simple linking molecules to particular ECM molecules. For example, there must be a reason for the different patterns of three known collagen receptors (VLA-1, -2, and -3); and VLA-5 is usually confined to the lumen of large vessels, while the other fibronectin receptor (VLA-3) is found both there and on extravascular tissue.

The apparent association of the α_6 chain with β_4 in the spleen is in stark contrast to the complete absence of β_4 in the kidney. This complex is specifically found at the base of the sinusoidal endothelial cells, which suggests that it has an equally specific function at this site. There could also be variation of β_4 expression between individuals, as Kajiji et al (1987) found no β_4 in the spleen using the same antibody (S3-41).

3.4 (5) The anti-VLA-2 monoclonal antibody RMAC11 inhibits HUVEC adhesion to collagen types I and IV and laminin

The monoclonal antibody RMAC11 was produced after immunisation of a BALB/c mouse with cultured HUVEC (O'Connell PhD thesis, University of Melbourne, 1989). It binds to an epitope on the VLA-2 α chain (α_2). The effects of RMAC11 on the adhesion of cultured HUVEC to various substrates were studied in short-term (2 hour) assays, performed in medium without serum to ensure that the HUVEC adhered directly to the substrate coated onto the base of the well, rather than using fibronectin in the serum as a linking molecule.

Cultured HUVEC adhere and spread well on purified fibronectin and collagen (types I and IV) in the absence of serum. Approximately 30% fewer cells adhere to laminin under these conditions, and they have a more spindly, less spread-out appearance. In the presence of serum, HUVEC adhere and spread well on all these substrates, as well as gelatin or plastic alone.

Saturating concentrations of RMAC11 reduce the adhesion of HUVEC to both types of collagen and laminin by 30 to 40%, but has no inhibitory effect on their adhesion to fibronectin (FN) (see graph page 152). The control antibodies (P3X63Ag8, the anti-HLA class I antibody W6/32, QE.2E5, and the anti-VLA-5 antibody PHM2 [see section 3.4 (6)]) had no effect on adhesion to collagen or laminin. There were not only fewer adherent cells with RMAC11, but they also had a rounded-up shape that was in marked contrast to the spread, irregular appearance of cells cultured on fibronectin or in the presence of the control antibodies.

Discussion

These results demonstrate that VLA-2 on HUVEC is a receptor for collagen (types I and IV) and laminin, and that RMAC11 binds to a functional epitope on the VLA-2 α chain. The antibody does not affect the adhesion and spreading of HUVEC on fibronectin. RMAC11 interferes with both cell spreading and adhesion to collagen and laminin, and the cells have a distinctive rounded-up appearance before they are washed off.

However, a significant percentage of cells remain adherent to the collagen and laminin despite the presence of RMAC11, although most of these were not as well spread out as cells coated with control antibody. There are several potential explanations for this incomplete inhibition. The washing step may be insufficient to remove all non-adherent cells, the RMAC11 may not completely block the VLA-2 receptor, or the HUVEC may possess other collagen and laminin receptors. Two other possible collagen receptors from the integrin β_1 family (VLA-1 and VLA-3) are also expressed by HUVEC, but the monoclonal antibodies used in these studies that bind to those receptors (TS2/7 and J143) bind to non-functional epitopes, and cannot be used to test that hypothesis.

Before these experiments were completed, a study was published that confirmed that VLA-2 is a collagen and laminin receptor on HUVEC (Languino et al, 1989). The inhibition of adhesion to collagen and laminin in that study was 80-90%, which may be due to different experimental conditions, or because their anti-VLA-2 antibody more completely blocked the receptor.

Laminin and collagen type IV (along with proteoglycans) are the predominant molecules that make up basement membranes.The fact that VLA-2 is a HUVEC receptor for both of these ECM proteins suggests that it attaches endothelial cells to their basement membranes in vivo. VLA-2 is a

collagen receptor on platelets and it is likely that RMAC11 would also inhibit platelet adhesion to collagen and collagen-induced platelet aggregation (not tested).

3.4 (6) The monoclonal antibody PHM2 inhibits cellular adhesion to fibronectin and binds to VLA-5

The monoclonal antibody PHM2 was originally described as a marker for monocytes and macrophages (Becker at al, 1981). It also binds to megakaryocytes, platelets, endothelial cells in a variety of tissues, fibroblasts, hepatic parenchymal and biliary tract cells, and 5-10% of T lymphocytes (Hancock et al, 1983). The following experiments show that PHM2 inhibits cellular adhesion to fibronectin and immunoprecipitates a complex that has the characteristics of the fibronectin receptor VLA-5.

Cultured HUVEC adhere and spread well on purified fibronectin, as do the cell lines U937 and K562. However, these two cell lines adhere poorly to other substrates, including collagen and laminin. The only member of the VLA family that K562 cells express in significant amounts is the fibronectin receptor VLA-5, whereas U937 also express a small amount of VLA-4 (Hemler et al, 1987b).

In cell-matrix adhesion experiments, the monoclonal antibody PHM2 inhibits the adhesion of cultured HUVEC to fibronectin by 30 to 40% in a concentration-dependent manner, but has no effect on their adhesion to collagen or laminin. Control antibodies (including the anti-VLA-2 antibody RMAC11), have no effect on HUVEC adhesion to fibronectin. Saturating concentrations of PHM2 completely block U937 and K562 adhesion to fibronectin, whereas control antibodies have no effect (see graph page 153). The known anti-VLA-5 monoclonal antibody B1E5 has comparable effects in parallel experiments. These results demonstrate that PHM2 inhibits fibronectin receptor function on these cells.

The original description of the cellular distribution of the antigen recognised by PHM2 is consistent with the known distribution of VLA-5, and the staining patterns in the kidney (photographs page 154) and spleen (not shown) of PHM2 and BIE5 (sections 3.4 (2) and 3.4 (4)) are identical.

PHM2 immunoprecipitates antigen poorly, possibly because of low affinity or because the epitope is disrupted during the procedure. It weakly immunoprecipitates from cultured HUVEC bands at 150 and 110 kD non-reduced, and a broad band at 130 to 140 kD under reducing conditions. This

is identical to the precipitation pattern obtained with BIE5 (photograph page 155), and is contained within the more complex bands precipitated by the anti-β_1 antibodies A-1A5 and QE.2E5 (section 3.2). However, attempted sequential immunoprecipitation studies with PHM2 and these antibodies using HUVEC or other cells have not been successful.

Discussion

Despite the incomplete immunoprecipitation data, there is strong evidence that PHM2 recognises the same antigen as BIE5 ie the fibronectin receptor VLA-5. They have identical cellular and tissue distributions, both specifically inhibit cellular adhesion to fibronectin, and PHM2 has the appropriate precipitation pattern from HUVEC for VLA-5. These studies do not reveal whether PHM2 binds to the α chain of VLA-5, or to a conformational epitope formed by the α_5 and β_1 chains.

PHM2 only partially inhibits HUVEC adhesion to fibronectin, probably because they also express VLA-3 and some VLA-4 as potential fibronectin receptors. This hypothesis could not be confirmed by further blocking studies, as the anti-VLA-3 and anti-VLA-4 antibodies available to us (J143 and B5G10) do not bind to functional epitopes. K562 only express significant amounts of VLA-5 as a potential fibronectin receptor, and therefore their adhesion to fibronectin is completely blocked by PHM2 and BIE5. However, U937 express some VLA-4 as well as VLA-5. The complete inhibition of U937 adhesion to fibronectin may be because their VLA-4 is not in a functional form, or that the fibronectin used in these experiments does not contain significant quantities of the alternatively spliced form that interacts with VLA-4.

The few anti-VLA-5 monoclonal antibodies described in the literature include the BIE5 used in this study, BIIG2 (Brown et al, 1989), PIF8 and PID6 (Wayner et al, 1988), and antibody 16 (Akiyama et al, 1989a). PHM2 binds to a functional epitope on VLA-5 , and is a useful addition to this short list.

3.4 (7) The VLA-6 α chain associates with the β_4 chain on cultured HUVEC

The VLA-6 α chain (α_6) associates with the β_4 chain on some epithelial cell lines (Sonnenberg et al, 1988a, Hemler et al, 1989). Antibodies against the β4 chain also only stain epithelial cells (normal and malignant) in tissue sections (Kajiji et al, 1989). The following studies show that the anti-VLA-6

monoclonal antibody GoH_3 immunoprecipitates a complex on cultured HUVEC that is distinct from $\alpha_6\beta_1$ and is probably the $\alpha_6\beta_4$ complex.

GoH_3 immunoprecipitates a complex from platelets that is composed of the α_6 and β_1 chains (Hemler et al, 1988) (photograph page 156). The pattern of precipitation is different using cultured HUVEC, and the strength of the bands (ie the level of expression of the α_6 chain) varies between HUVEC cell lines. Under non-reducing conditions, a distinct band at 135 kD (which corresponds to the α_6 chain) is co-precipitated with three other bands at 190, 155, and 115 kD. This pattern is clearly distinguishable from that obtained from the same cells with the anti-β_1 antibodies goat 172 and QE.2E5 (photograph page 156). Some of the α_6 chains may also be associated with β_1 chains, as the lower molecular weight band overlaps with the band containing the β_1 chains in the anti-β_1 antibody lanes.

Following complete preclearing of β_1 chains from a HUVEC lysate by the antibody A-1A5, the α_6 chain is still precipitated by GoH_3, although possibly with slightly decreased intensity. There are no additional higher molecular weight bands, but there is a broad, strong band at 115 kD (photograph page 134).

A comparative immunoprecipitation from HUVEC of GoH_3 and the anti-β_4 antibody 439-9B was performed, but the expression of the α_6 chain on these particular HUVEC cells must have been low as the bands are very weak. However, after prolonged autoradiography, an α_6 plus the three additional bands are just visible in the GoH_3 lane, and a similar complex is just visible in the 439-9B lane. However, these weak bands could not be photographed.

In an attempt to confirm that these bands represent the β_4 chain, other HUVEC were studied by ELISA and by flow cytometric analysis, using the anti-β_4 monoclonal antibodies S3-41 and 439-9B. Two separate HUVEC cell lines failed to bind either antibody by ELISA. However, three different HUVEC lines were very weakly positive by flow cytometric analysis (see page 145). Unfortunately, the lines which were used for the immunoprecipitation studies were not available for this analysis of their cell surface molecules.

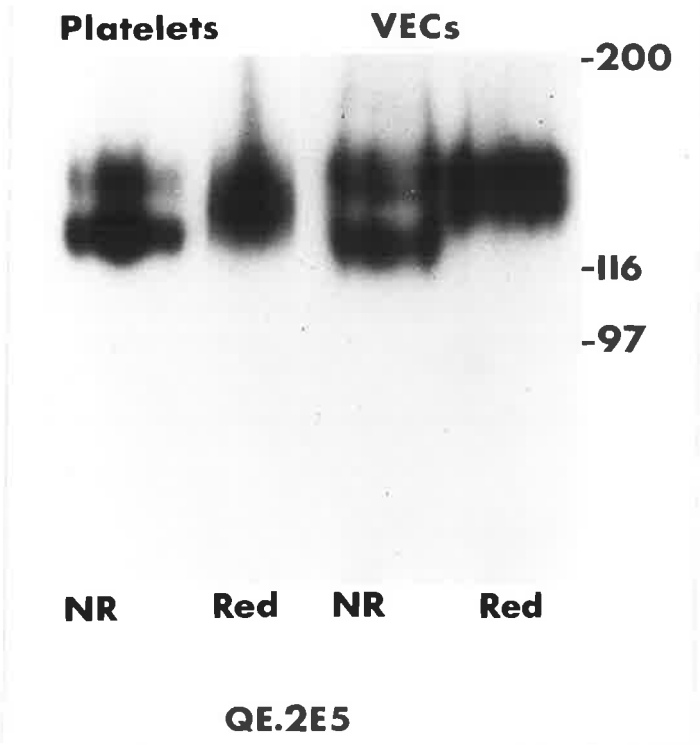
Discussion

The immunoprecipitation studies show that the α_6 chain expressed by some cultured HUVEC lines is co-precipitated with a group of molecules that resemble the integrin β_4 chains, as the molecular weights of these bands correspond to those described for the β_4 complex (Kajiji et al, 1989).

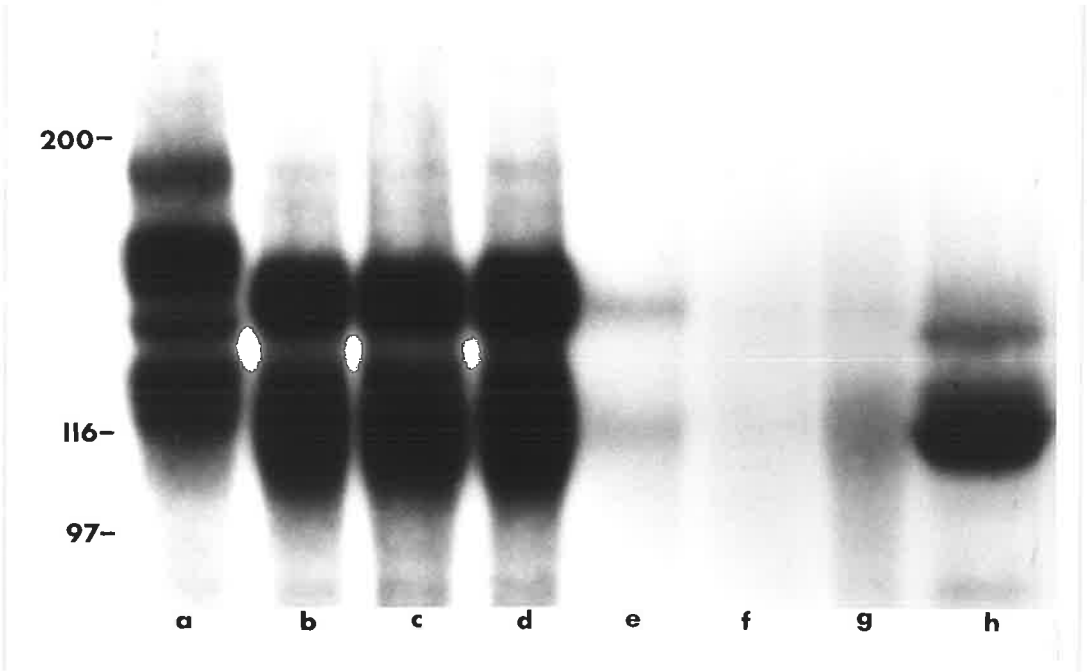
The dense lower molecular weight band that is co-precipitated with the α_6 chain following preclearing by the anti-β_1 monoclonal antibody A-1A5 suggests that the multiple chains have all been degraded during the procedure to a lower molecular weight form. This single band could still be consistent with the β_4 molecule, as the various molecular weight forms of the β_4 chains can be degraded to the lower molecular weight forms by neuraminidase digestion (Kajiji et al, 1989), and a similar degradation may have occurred during the immunoprecipitation procedure. The fact that the α_6 chain band is almost unaffected by preclearing with the anti-β_1 antibody shows that little if any α_6 was associated with the β_1 subunit.

While the evidence from the immunoprecipitation studies is quite convincing that the α_6 chains are complexed with β_4 chains on HUVEC, the information obtained from the ELISA and flow cytometric analysis does not fully support this observation. At best, the flow cytometric analysis (which is probably more sensitive than the ELISA) shows low expression of the β_4 chains on those particular HUVEC lines. One possible explanation for this difference is that some HUVEC lines express little or no β_4, and that their α_6 chains are complexed with β_1 chains. Alternatively, the bands precipitated with the α_6 chains could represent a novel integrin complex.

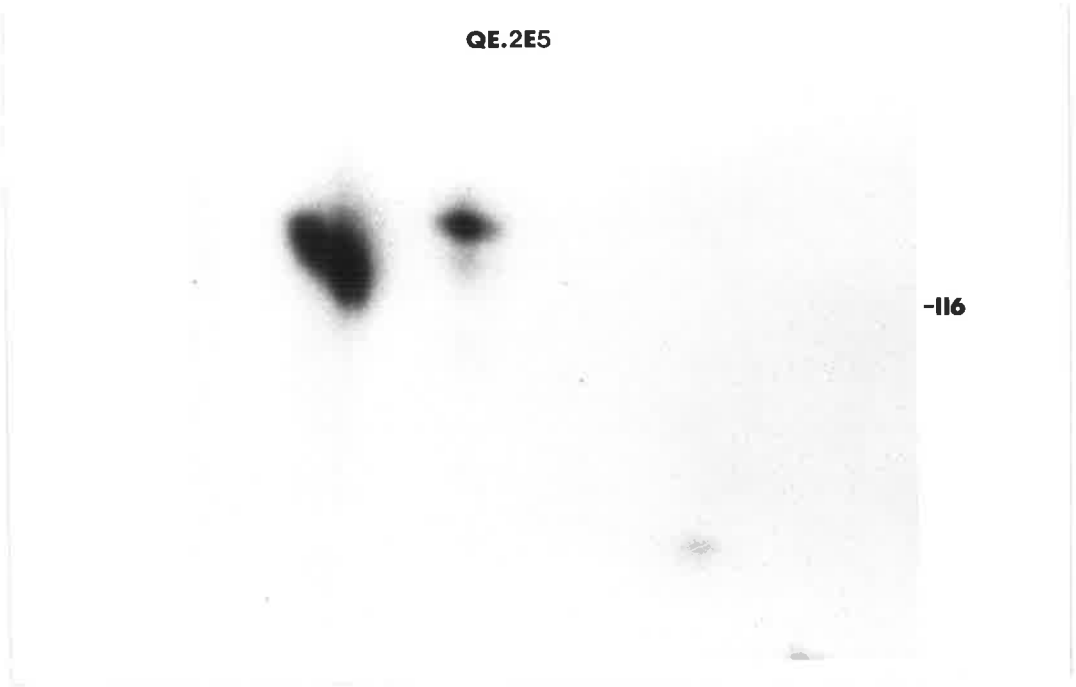
This observation is in some agreement with another study (Hemler et al, 1989) which showed that α_6 associated with some β_4 but mainly β_1 in an endothelial cell culture. In addition, the studies in section 3.3 (4) show that β_4 chains are expressed by the endothelial cells that line splenic sinusoids. However, other endothelium in the spleen and blood vessels in the kidney do not express β_4, although some of these cells express the α_6 chain (presumably associated with β_1).



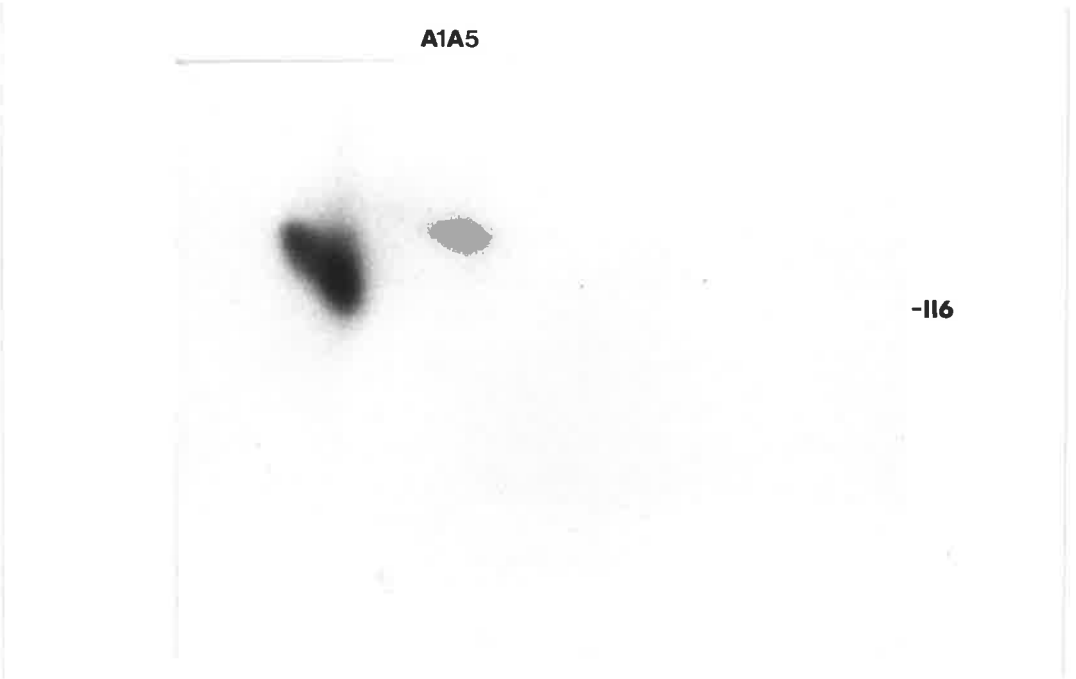
One-dimensional SDS-PAGE analysis of complexes immunoprecipitated by QE.2E5 from platelets and HUVEC (NR - non-reduced; Red - reduced; 7.5% gel)



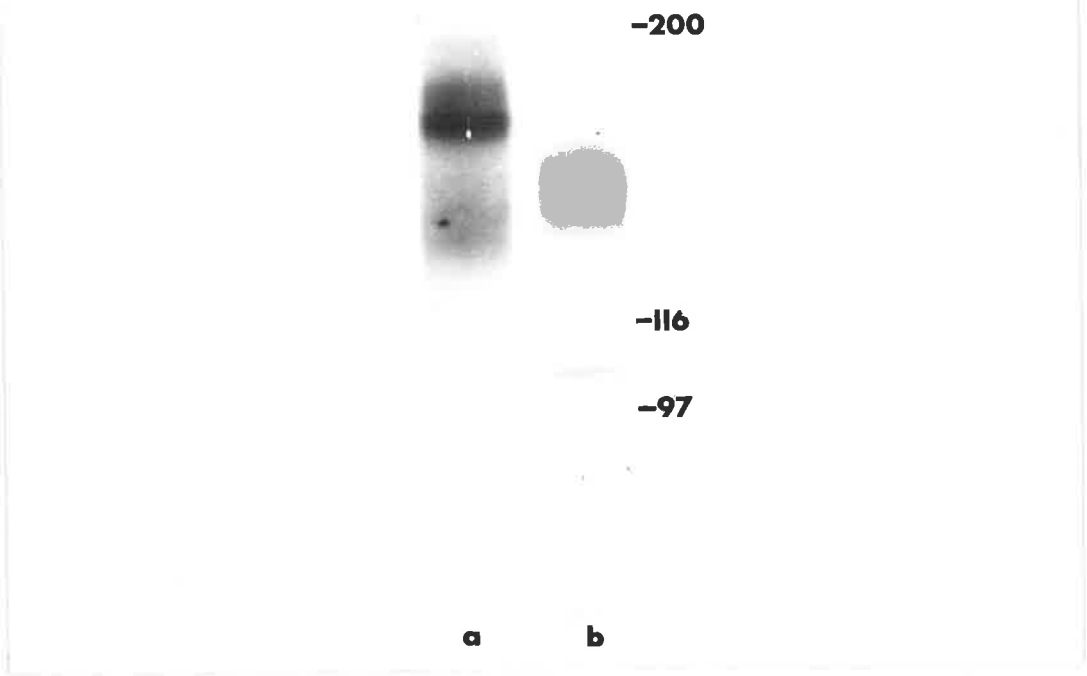
One-dimensional SDS-PAGE (non-reduced) analysis of HUVEC antigens: (a) GoH3 (anti-α_6) (b) QE.2E5 (c) goat 172 (anti-β_1 antiserum) (d) A-1A5 (e) A-1A5 - second preclearing of lysate (f) A-1A5 - fourth preclearing of lysate (g) goat 172 after fourth preclearing by A-1A5 (h) GoH3 after fourth preclearing by A-1A5 (5% gel)



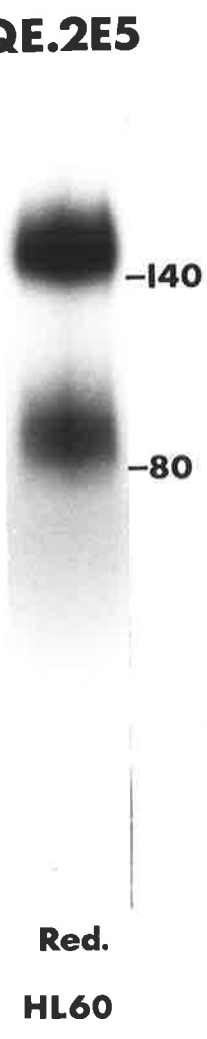
Isoelectric focussing followed by SDS-PAGE (non-reduced) in the second dimension; pattern immunoprecipitated by QE.2E5 from cultured HUVEC



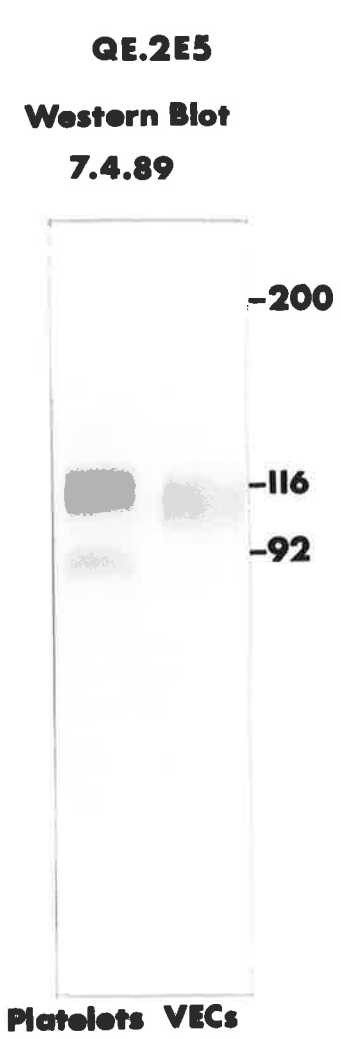
Isoelectric focussing followed by SDS-PAGE (non-reduced) in the second dimension; pattern immunoprecipitated by A-1A5 from cultured HUVEC



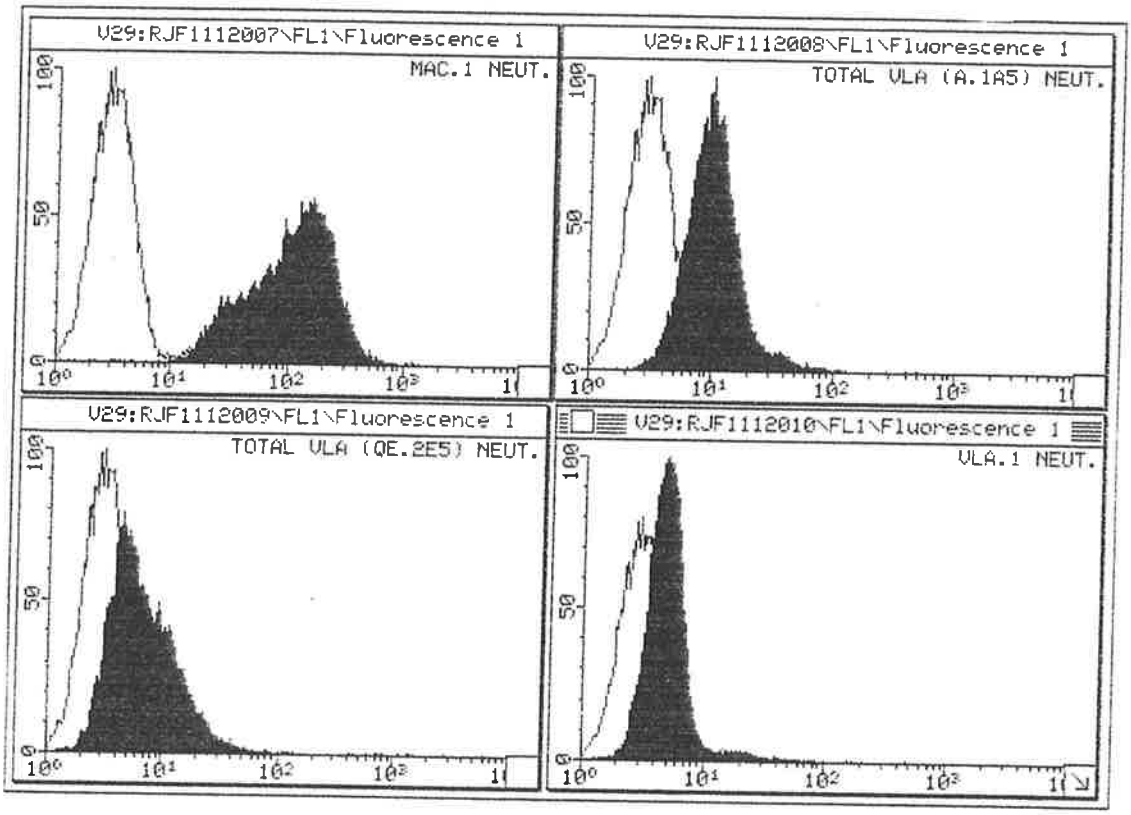
U937 - immunoprecipitation pattern obtained with QE.2E5 (a - non-reduced; b - reduced; 7.5% gel)

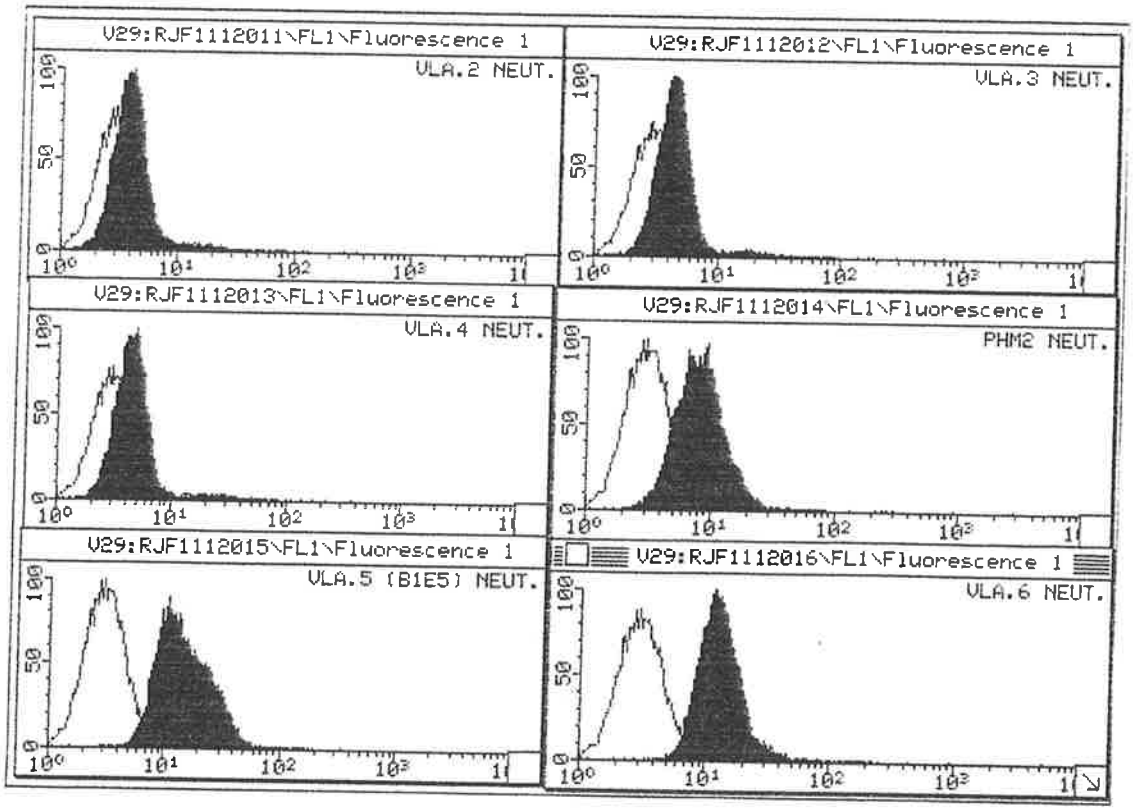


HL60 - immunoprecipitation pattern obtained with QE.2E5 (reduced; 7.5% gel)

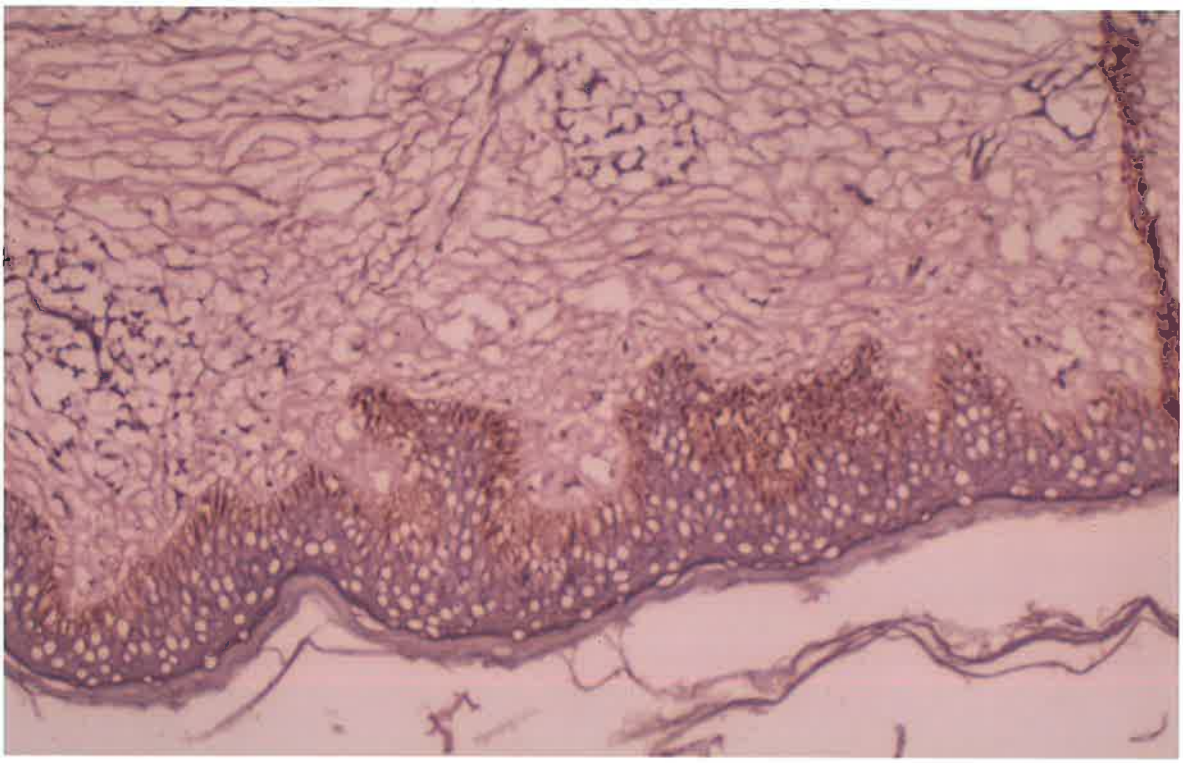


"Western" blot: bands recognised by QE.2E5 from platelet and HUVEC lysates (non-reduced)

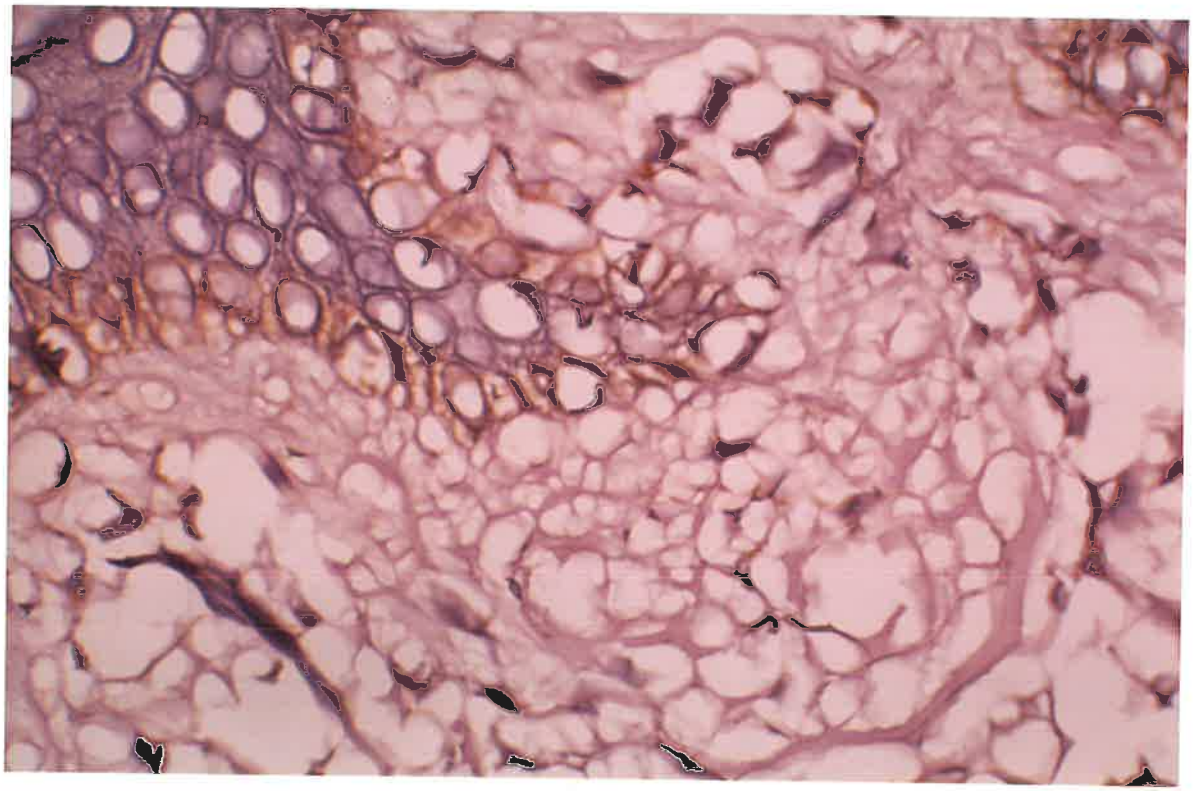




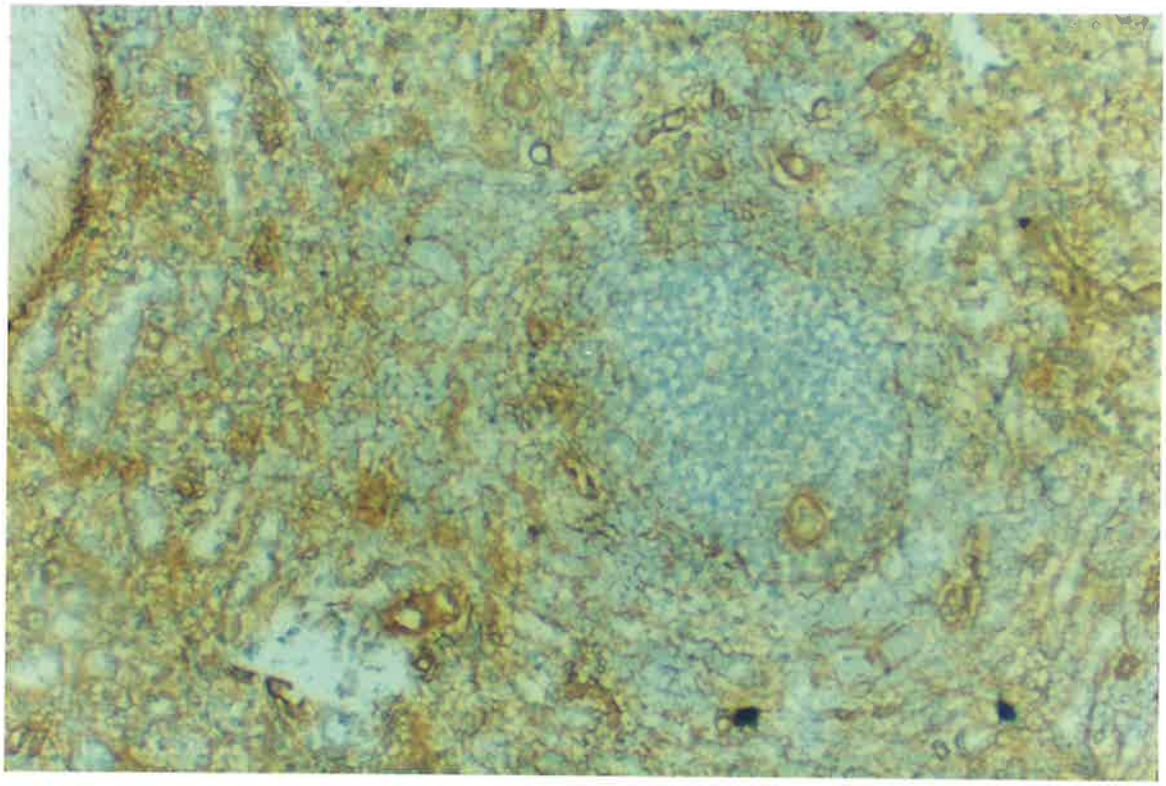
Flow cytometric analysis of neutrophil expression of QE.2E5 antigen, plus A-1A5 (β_1), α chains of VLA family, and PHM2 antigen (section 3.4 (6))



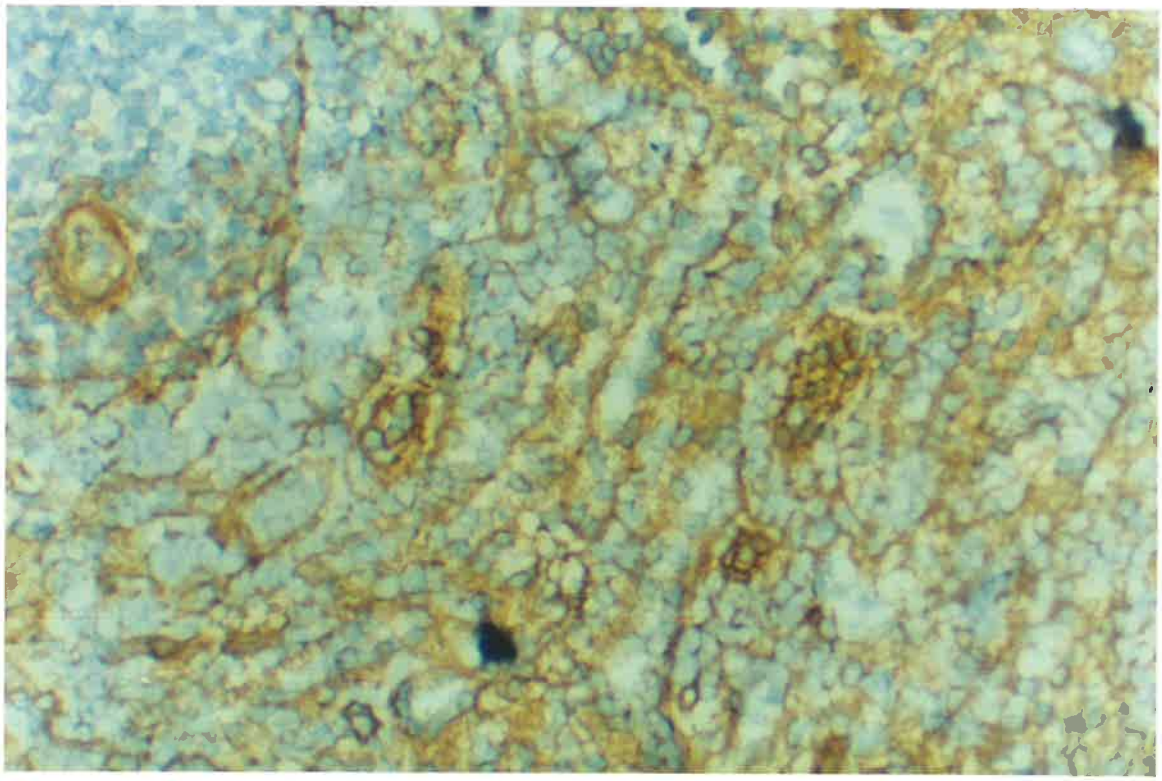
Expression of QE.2E5 antigen in normal human skin (indirect immunoperoxidase, x100)



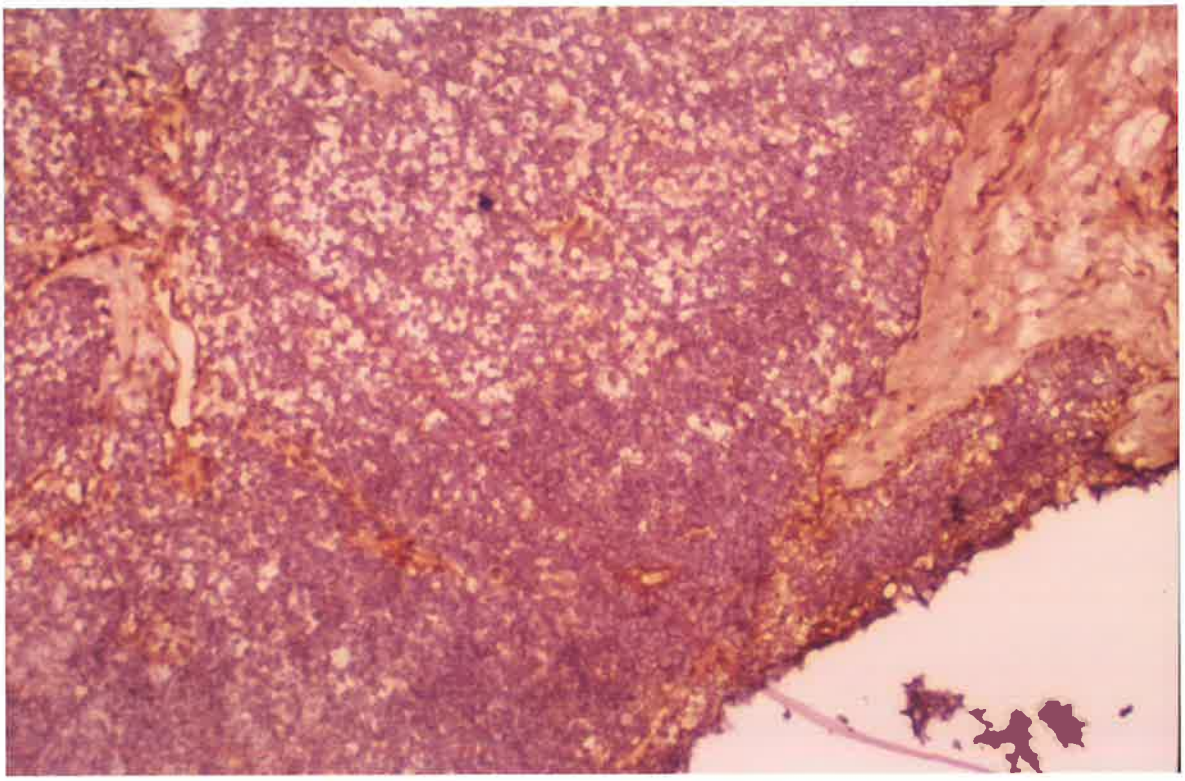
Expression of QE.2E5 antigen in normal human skin (indirect immunoperoxidase, x400)



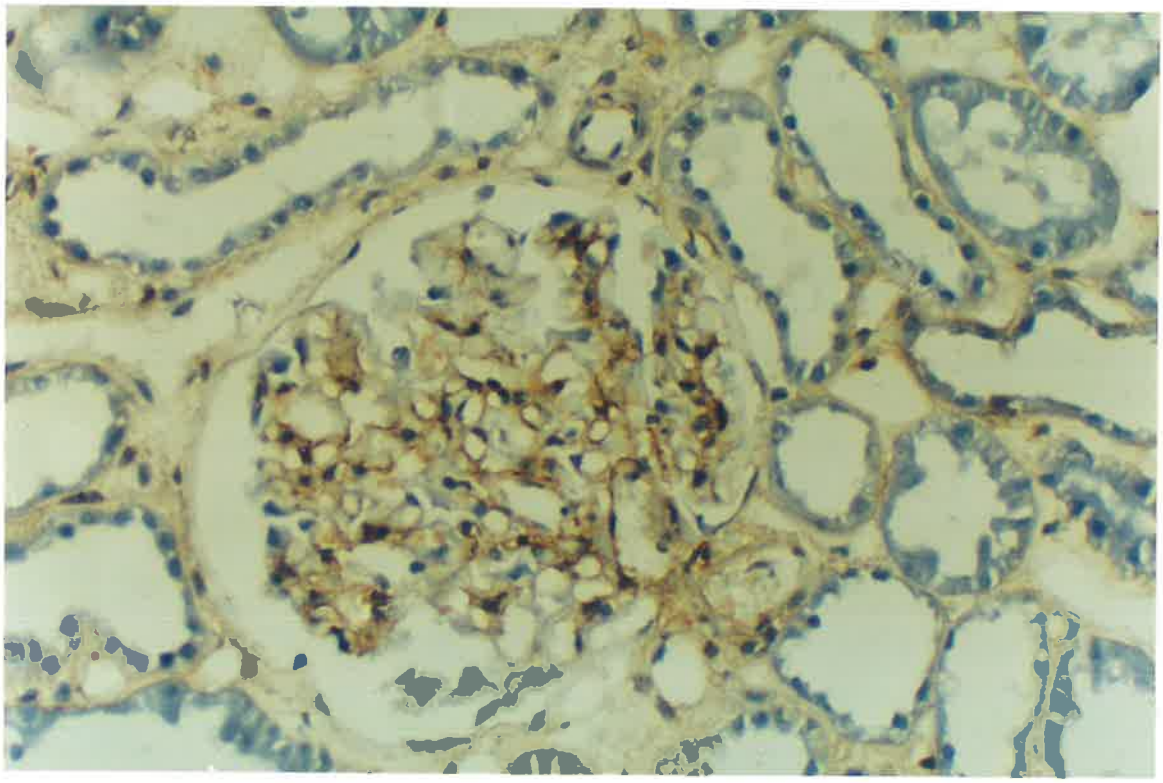
Expression of the QE.2E5 antigen in normal human spleen (indirect immunoperoxidase, x100)



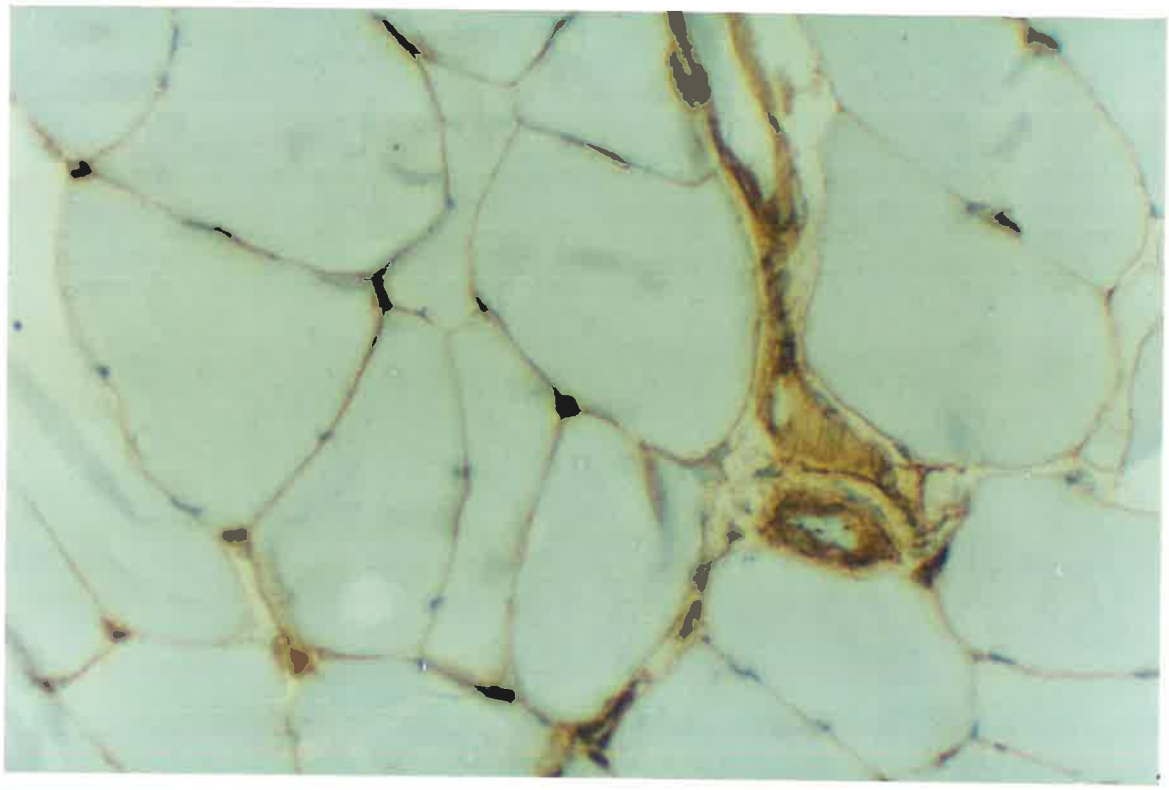
Expression of QE.2E5 antigen in normal human spleen (indirect immunoperoxidase, x200)



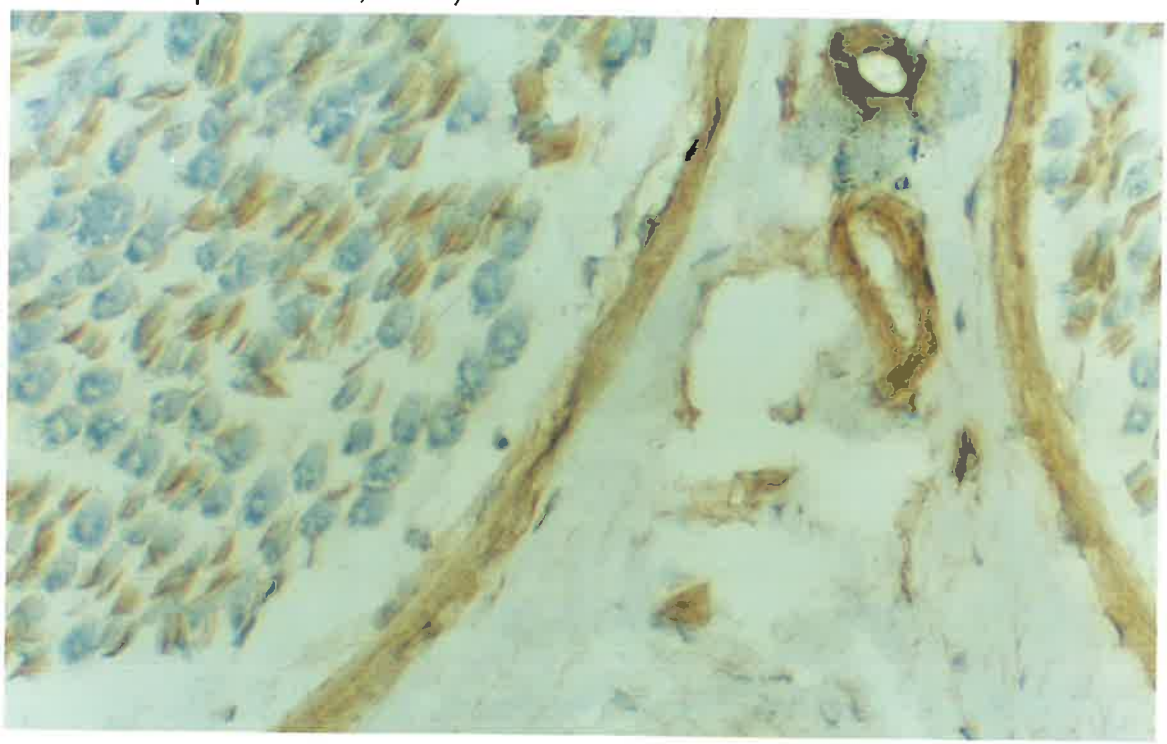
Expression of QE.2E5 antigen in normal human lymph node (indirect immunoperoxidase, x100)



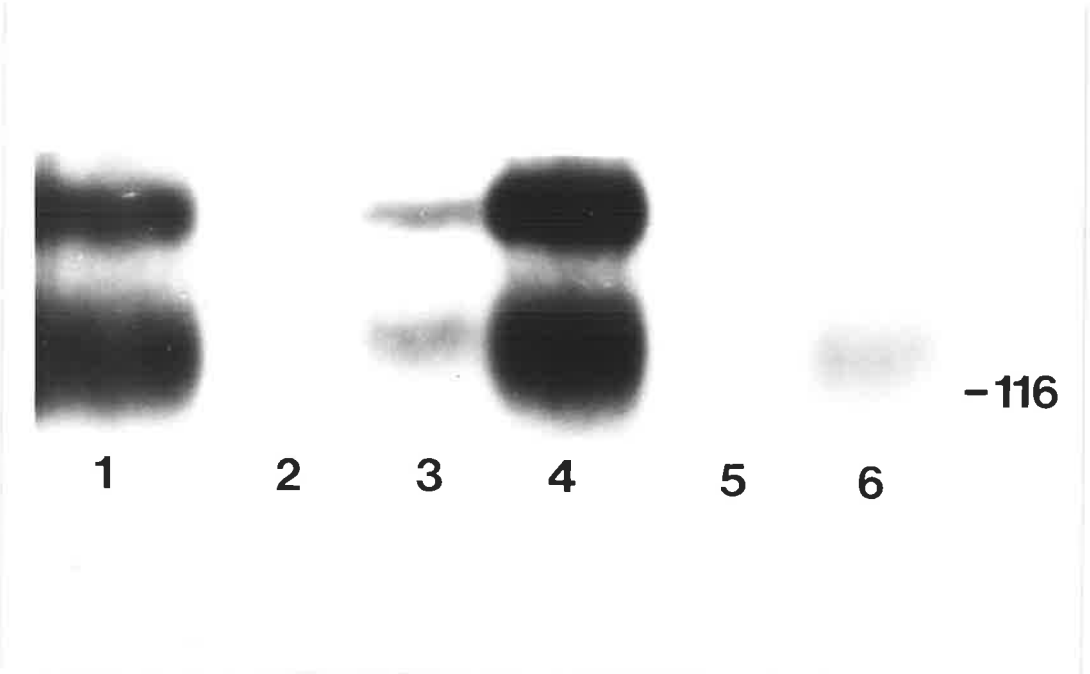
Expression of QE.2E5 antigen in normal human kidney (indirect immunoperoxidase, x200)



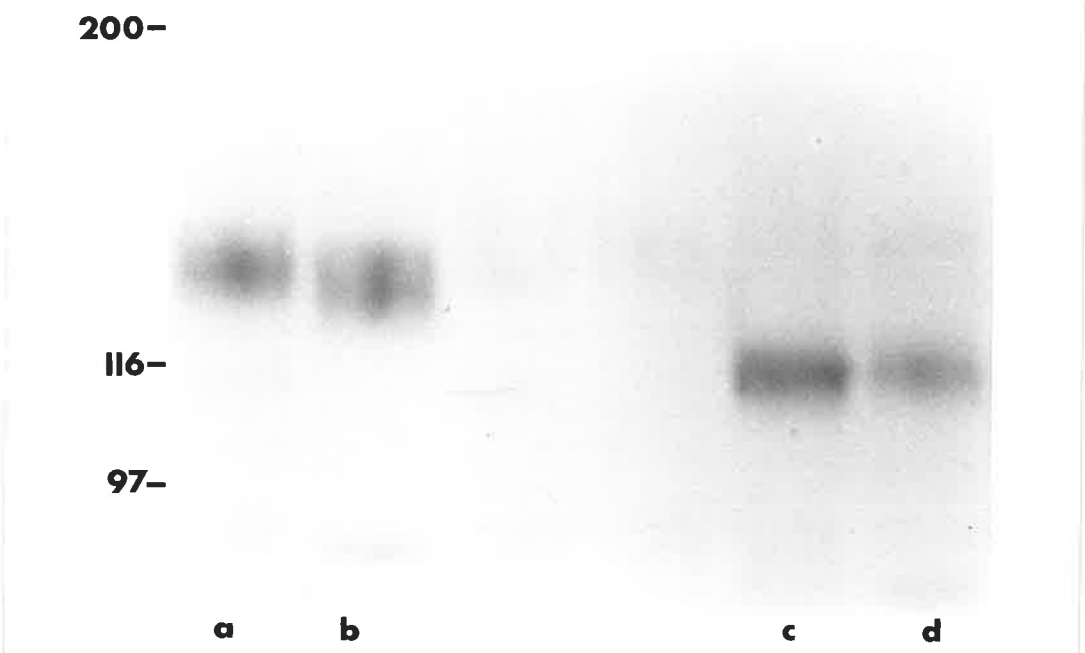
Expression of QE.2E5 antigen in normal human skeletal muscle (indirect immunoperoxidase, x200)



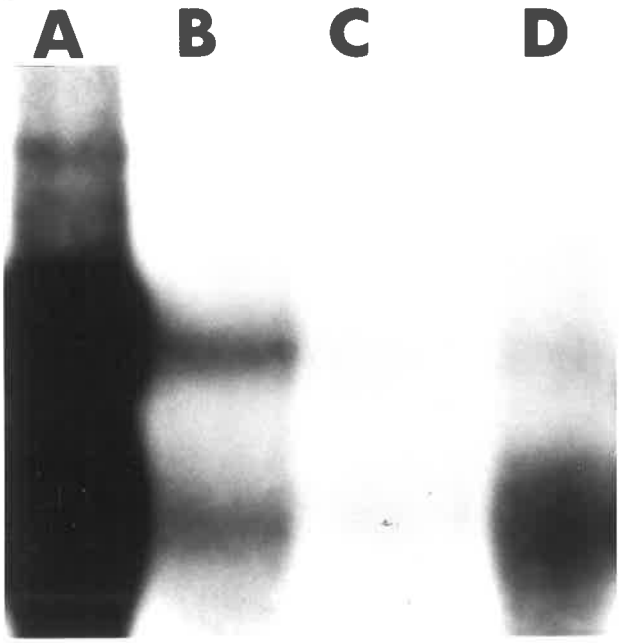
Expression of QE.2E5 antigen in peripheral nerve (tomaculous neuropathy) (indirect immunoperoxidase, x200)



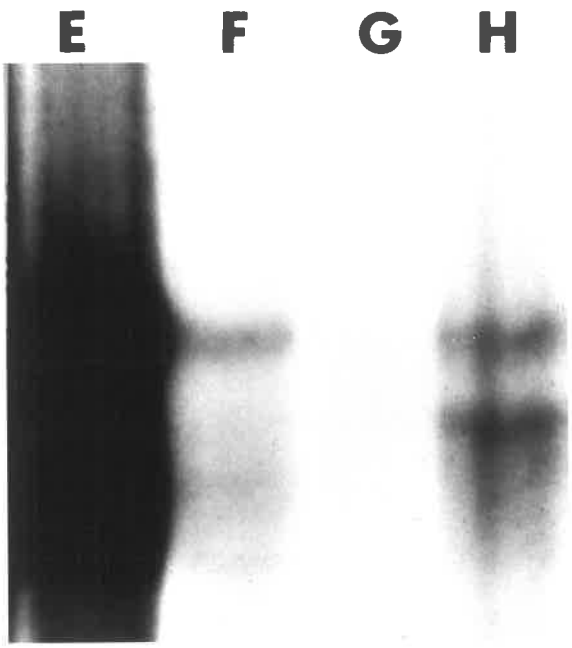
Sequential immunoprecipitation of platelet lysate (non-reduced): (1) QE.2E5 (2) lysate completely precleared of QE.2E5 antigen, followed by (3) A-1A5 (4) A-1A5 (5) lysate completely precleared by A-1A5 antigen, followed by (6) QE.2E5 (5% gel)



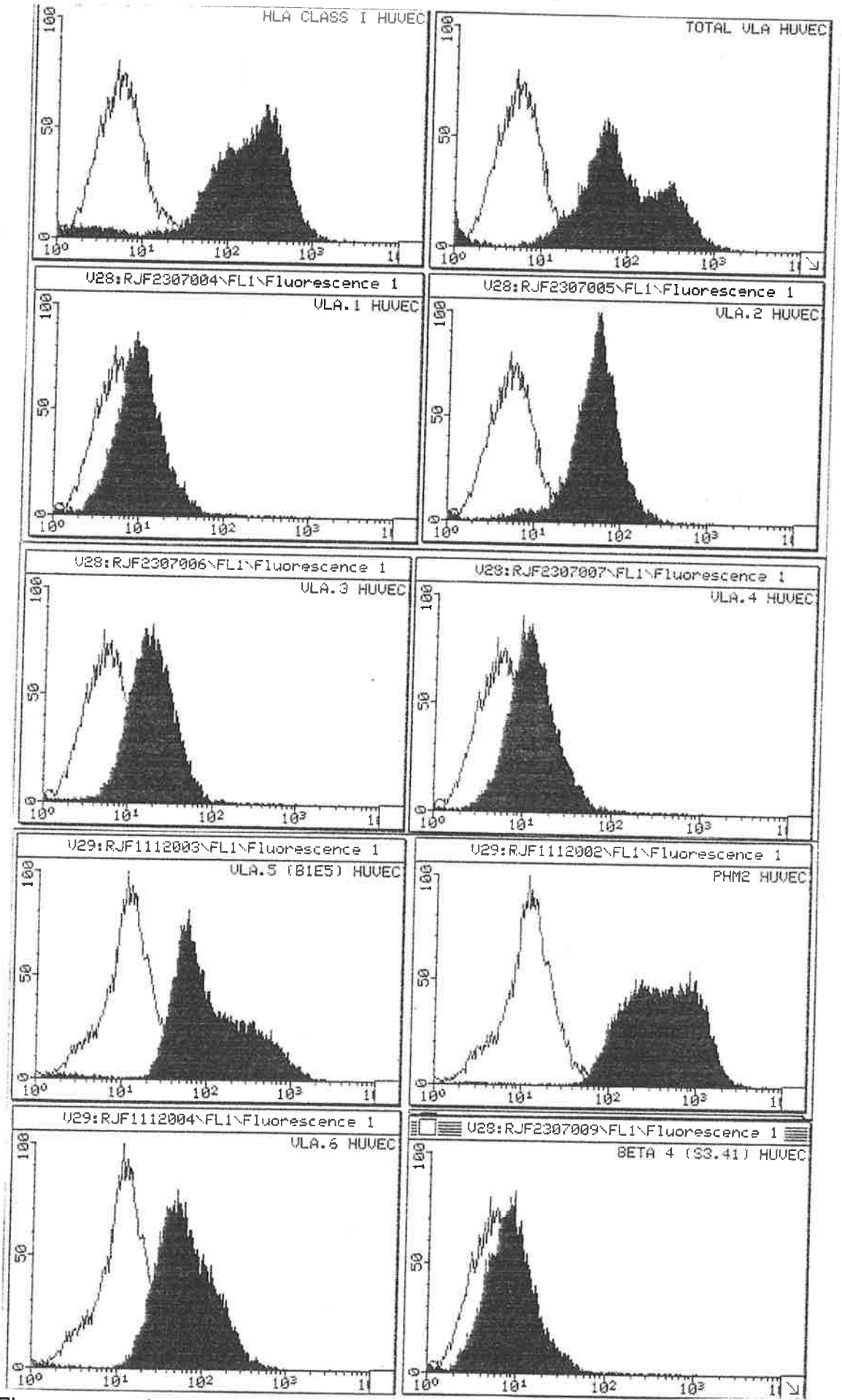
Comparative immunoprecipitation of QE.2E5 and A-1A5 using JM cells: (a) QE.2E5 reduced (b) A-1A5 reduced (c) QE.2E5 non-reduced (d) A-1A5 non-reduced (5% gel)



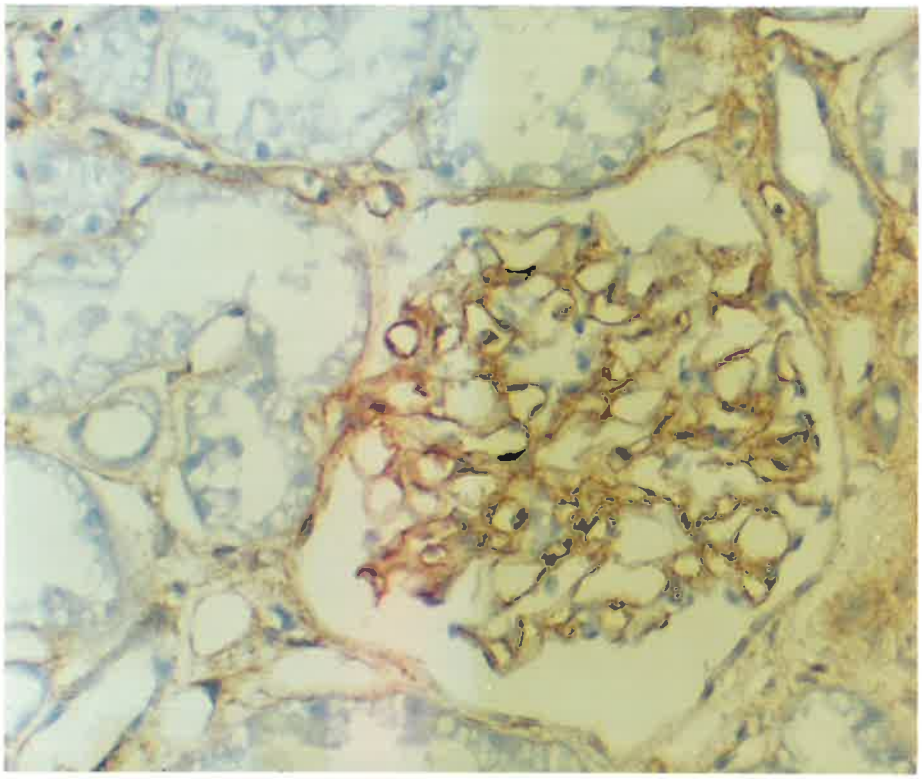
Sequential immunoprecipitation of HUVEC lysate with A-1A5 and goat 172: (A) A-1A5 (B) A-1A5 second immunoprecipitation (C) A-1A5 fourth immunoprecipitation, followed by (D) goat 172 (non-reduced, 5% gel)



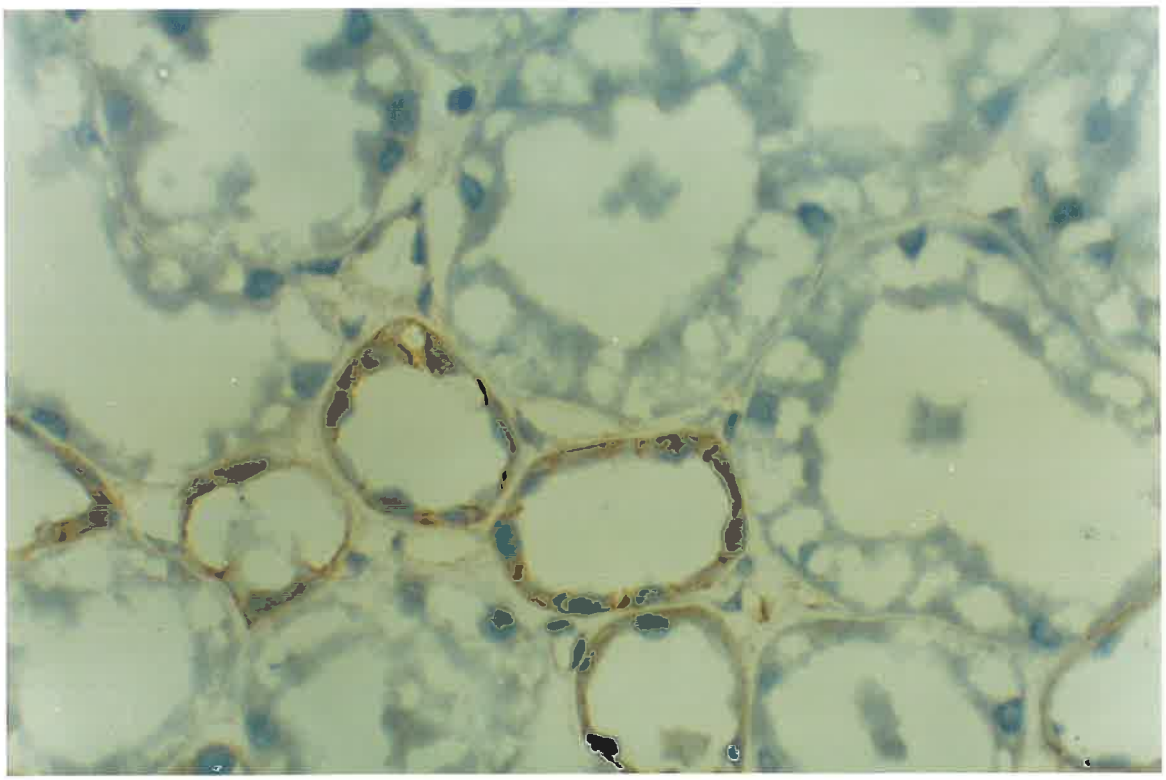
Sequential immunoprecipitation of HUVEC lysate with QE.2E5 and goat 172: (E) QE.2E5 (F) QE.2E5 second immunoprecipitation (G) QE.2E5 fourth immunoprecipitation, followed by (H) goat 172 (non-reduced, 5% gel)



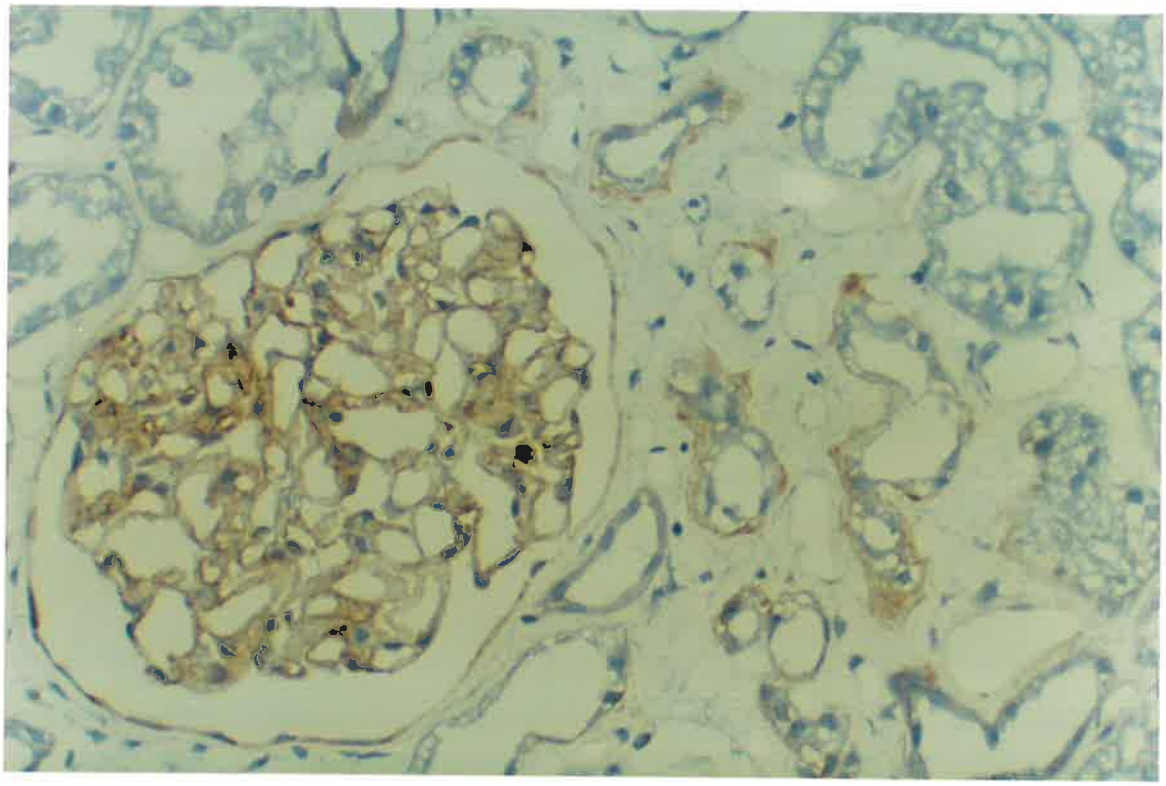
Flow cytometric analysis of VLA molecules expressed by cultured HUVEC (plus PHM2 (section 3.4 (6)) and β_4 chain)



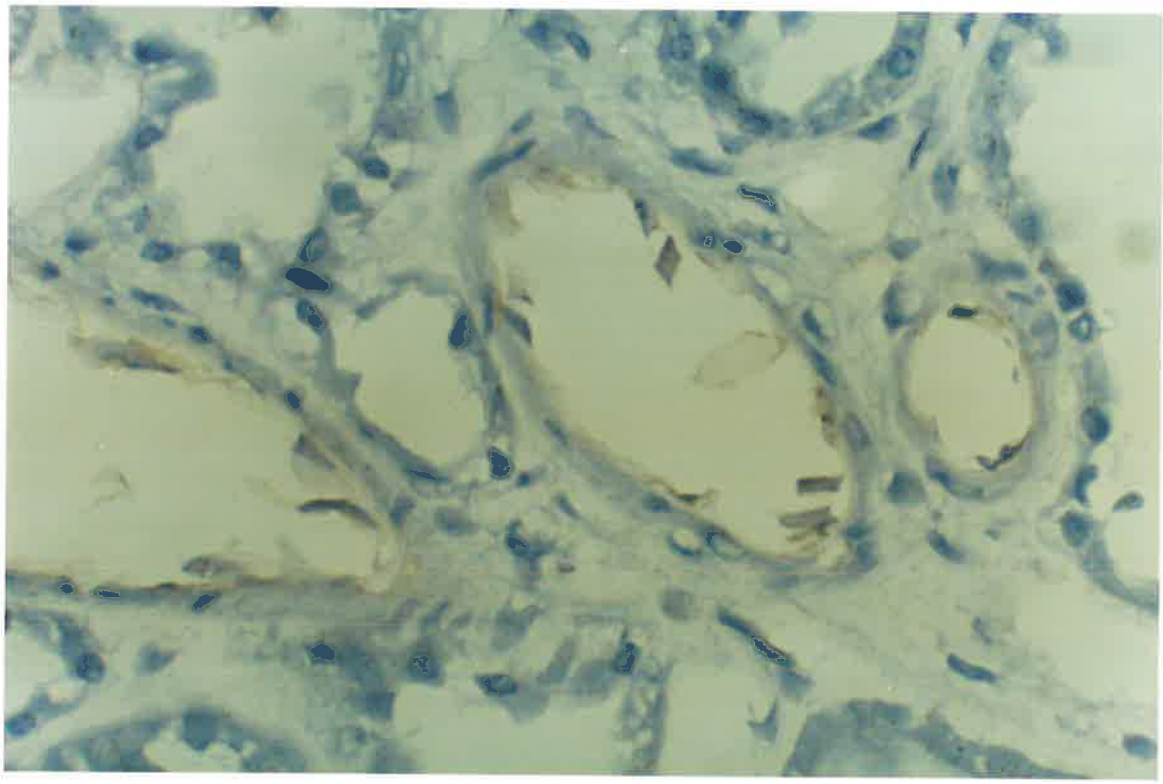
Expression of β_1 integrins (A-1A5) in normal human kidney (indirect immunoperoxidase, x200)



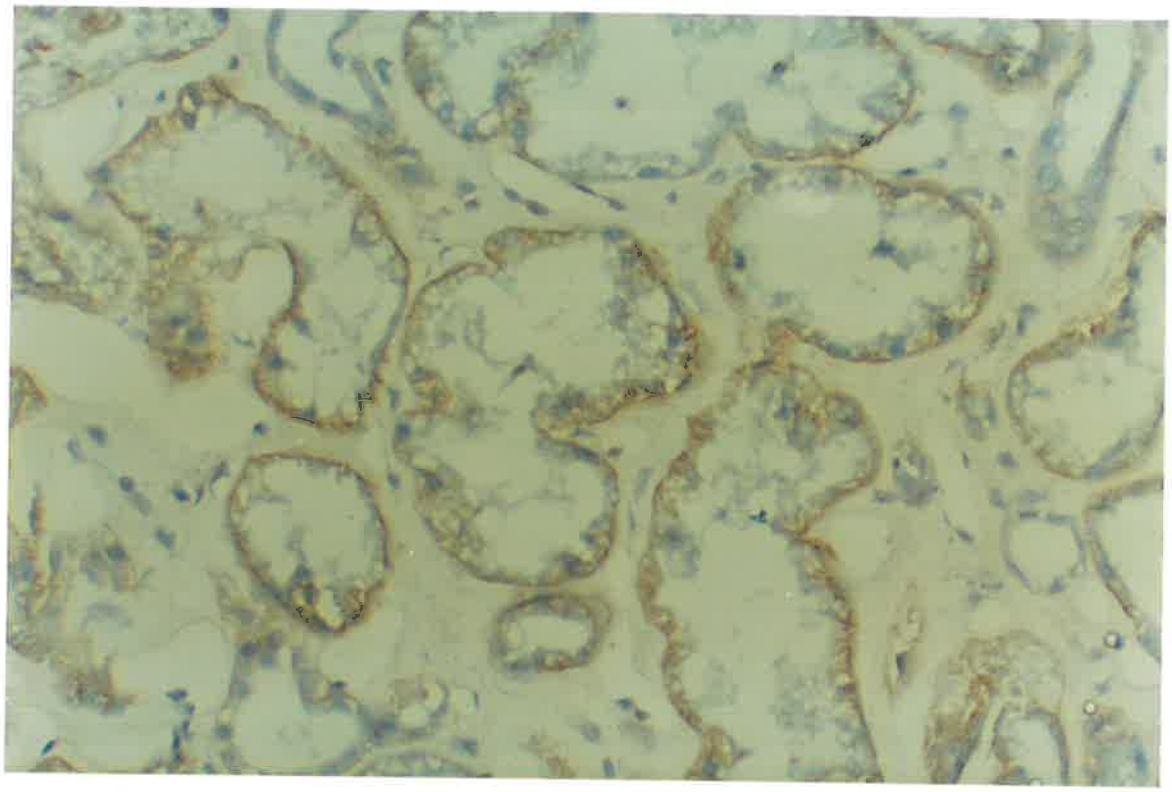
Expression of VLA-2 (RMAC11) in normal human kidney: staining of distal tubules (indirect immunoperoxidase, x400)



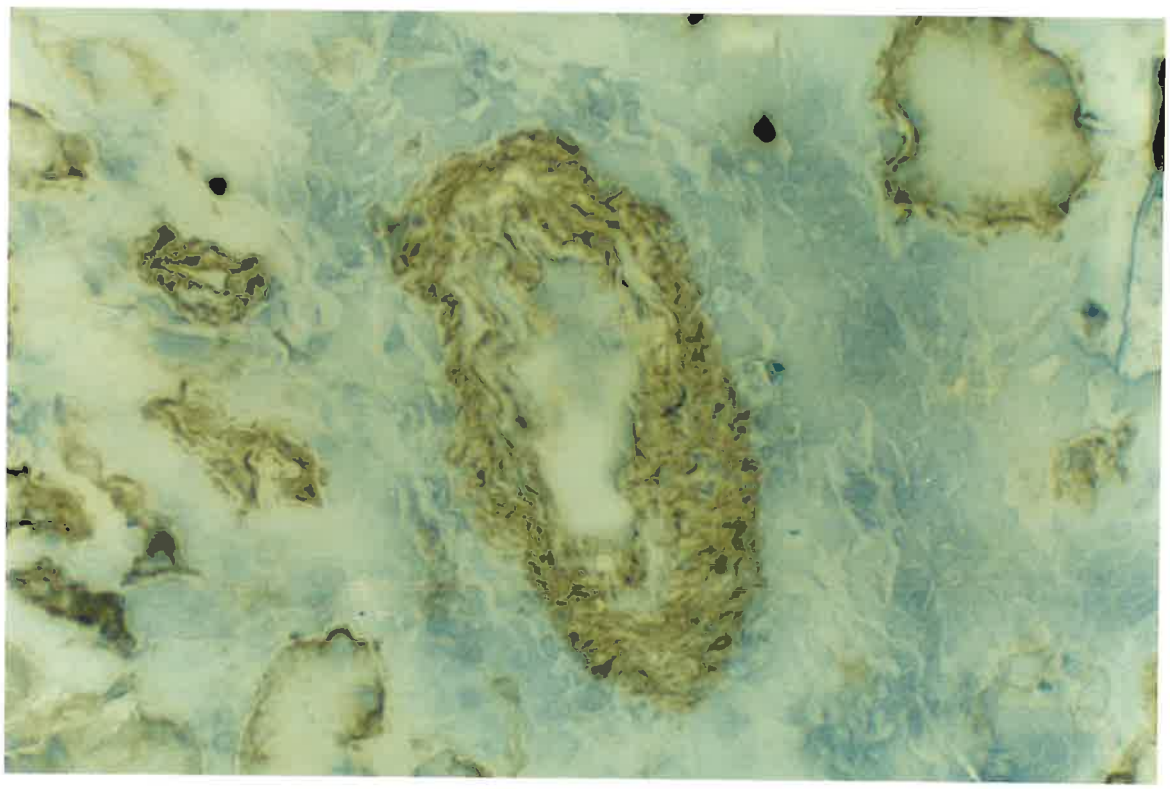
Expression of VLA-3 (J143) in normal human kidney (indirect immunoperoxidase, x200)



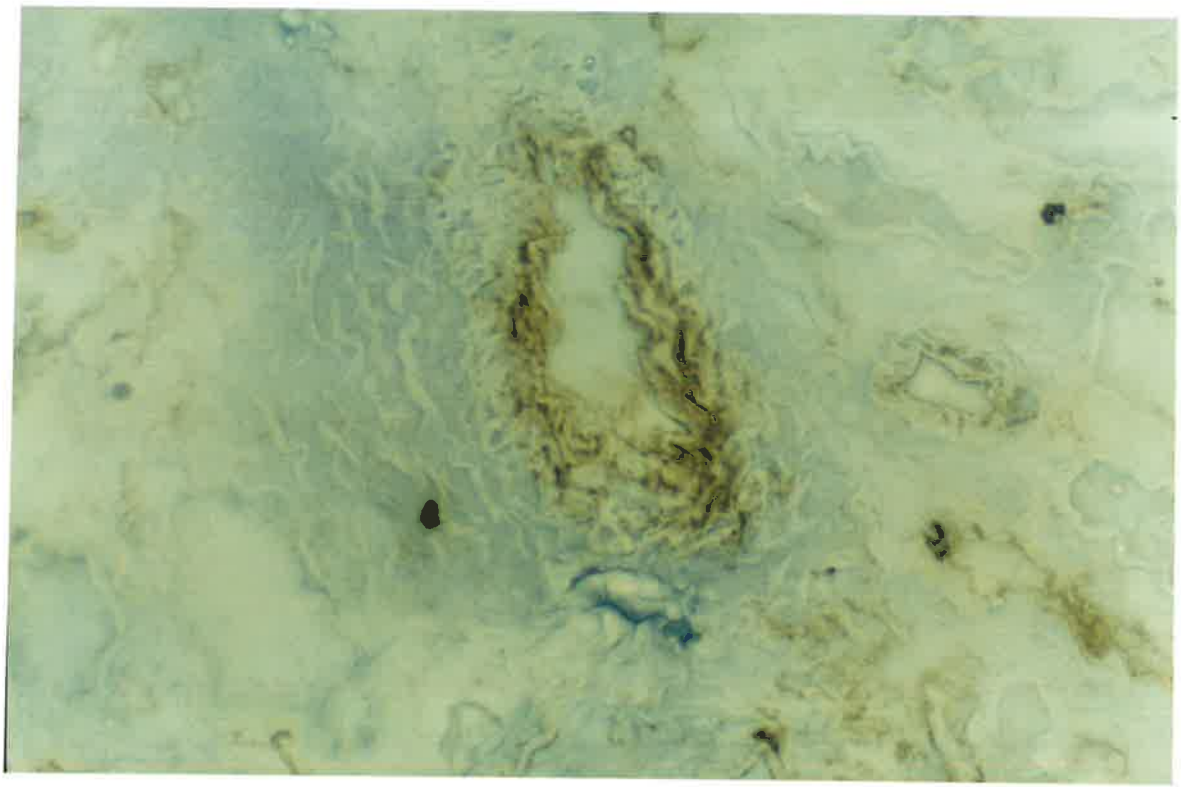
Expression of VLA-5 (B1E5) in normal human kidney: staining of intima of arterioles (indirect immunoperoxidase, x400)



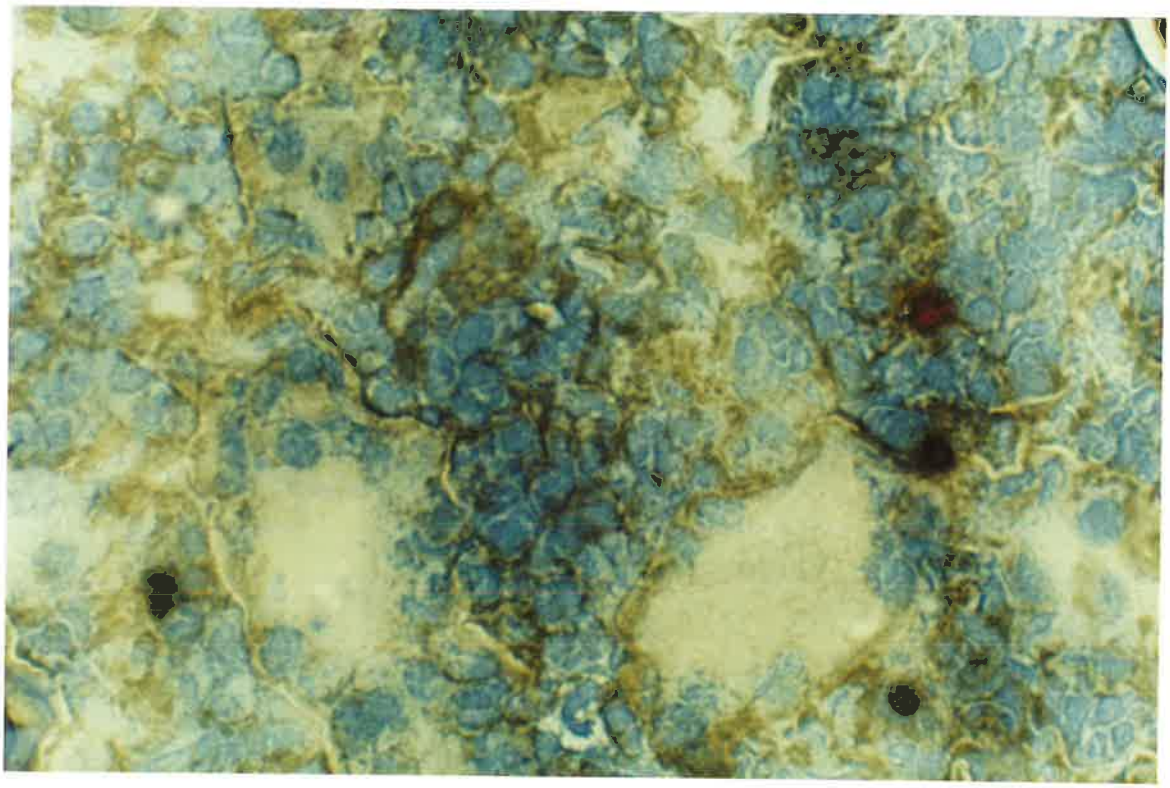
Expression of VLA-6 (GoH3) in normal human kidney: staining of the base of all tubules (indirect immunoperoxidase, x200)



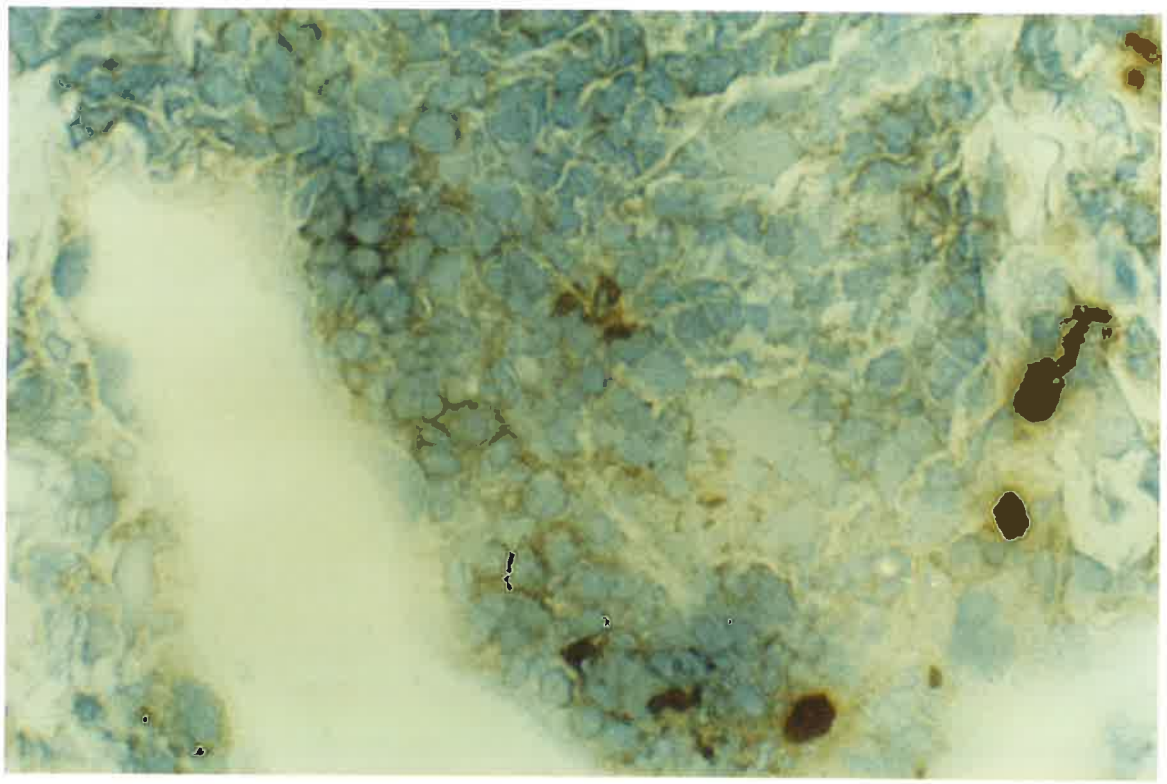
Expression of VLA-3 (J143) in rejecting renal allograft: strong staining of artery involved in vascular rejection (indirect immunoperoxidase, x200)



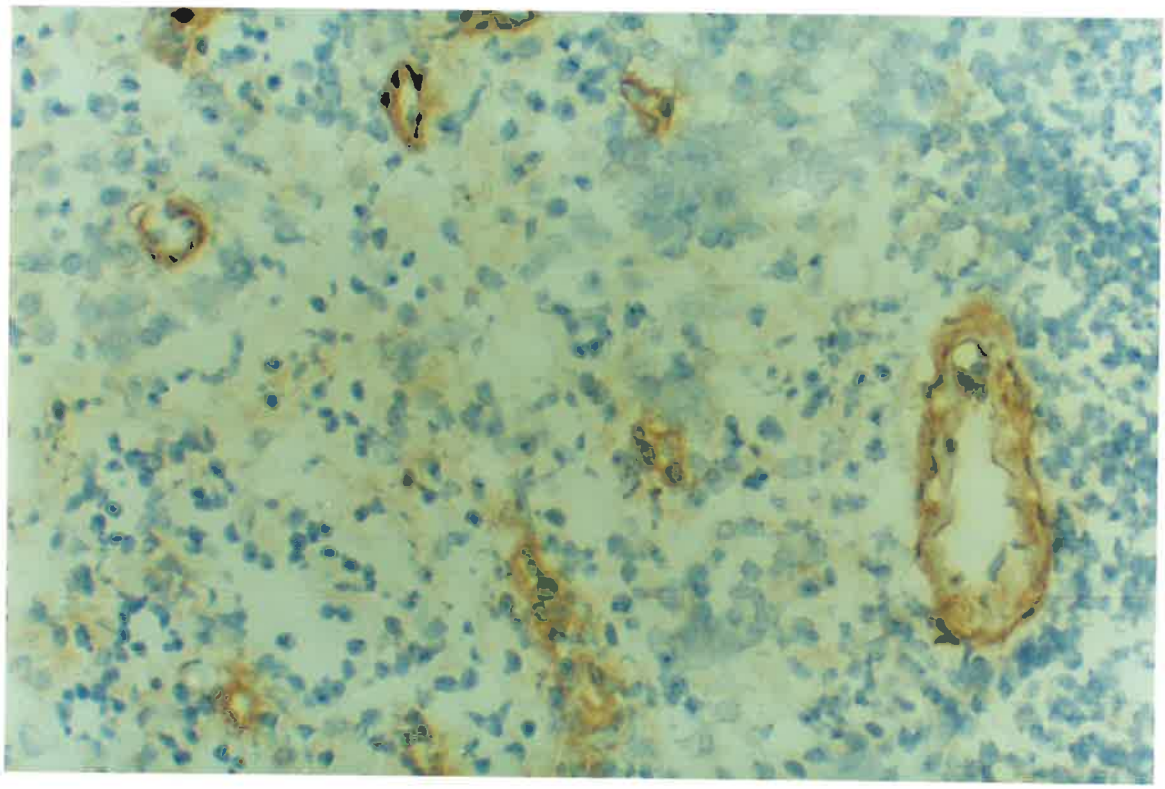
Expression of VLA-5 (PHM2 - see section 3.4 (6)) in rejecting renal allograft: strong staining of the intima of an artery involved in vascular rejection (indirect immunoperoxidase, x200)



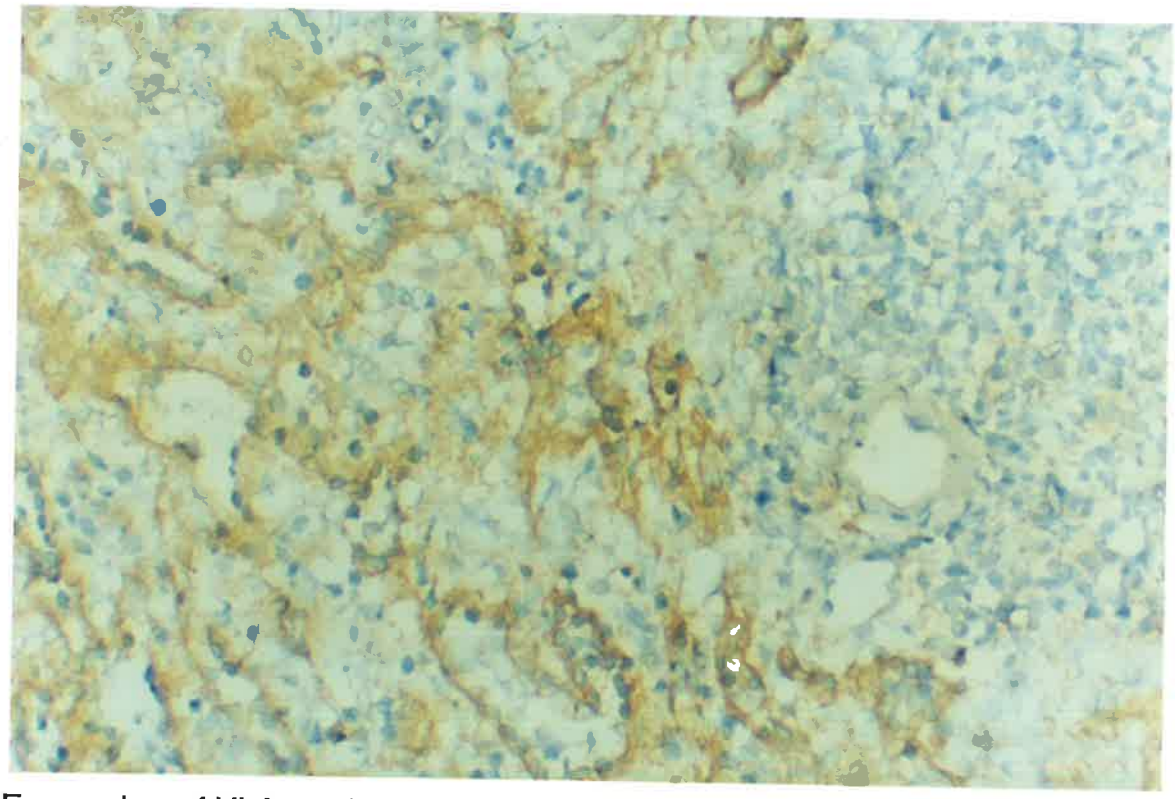
Expression of VLA-1 (TS2/7) on infiltrating cells in renal allograft rejection (indirect immunoperoxidase, x400)



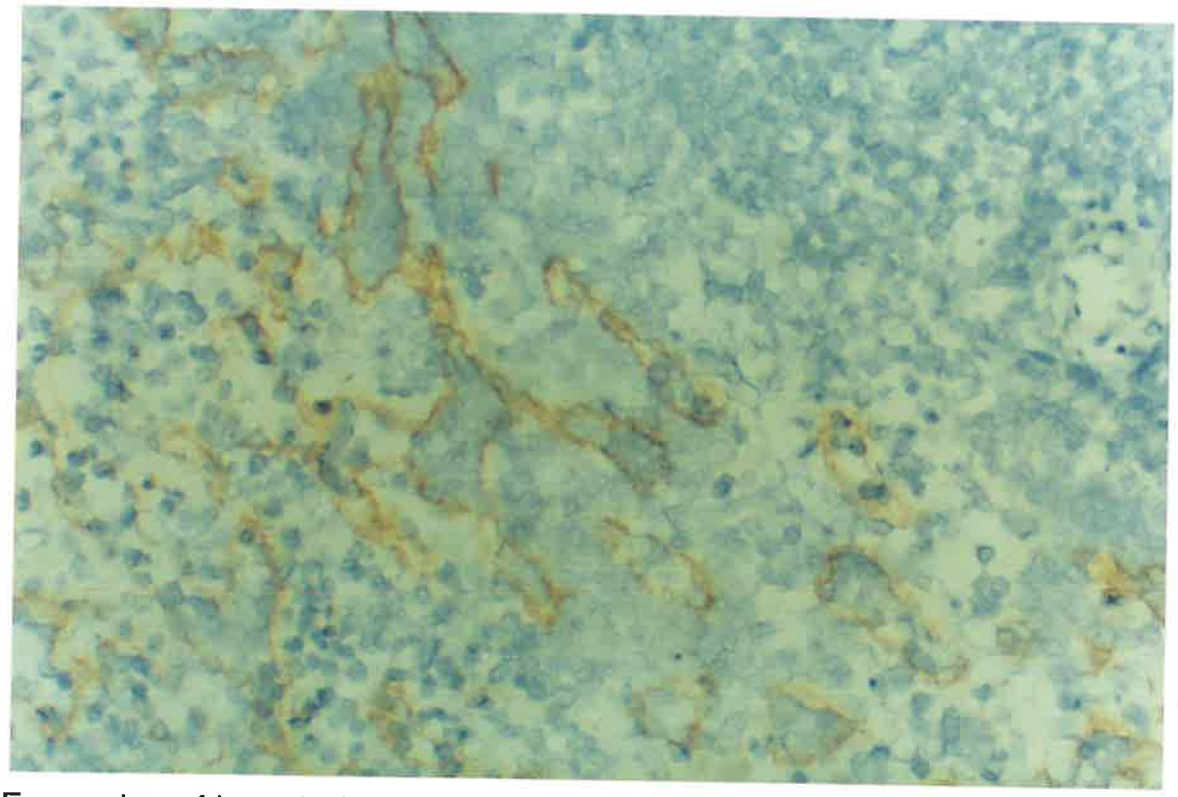
Expression of VLA-4 (B5G10) on infiltrating cells in renal allograft rejection; note that fewer cells are positive with increased distance from the vessel (indirect immunoperoxidase, x400)



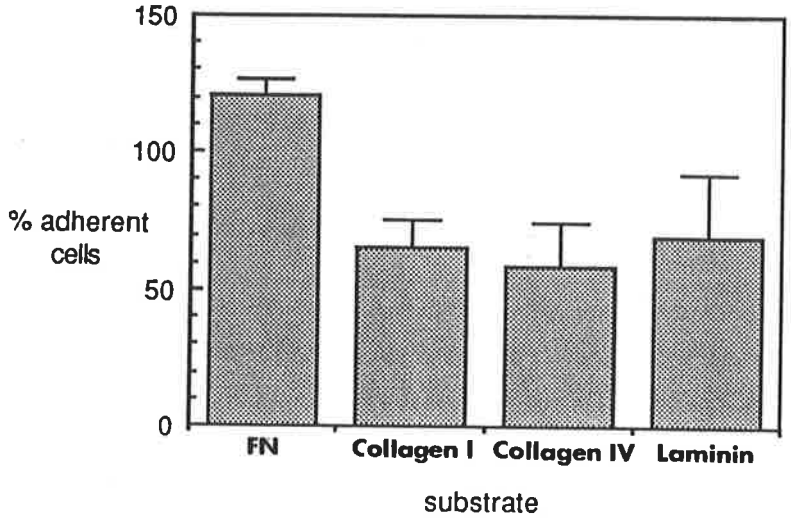
Expression of VLA-3 (J143) in the normal human spleen (indirect immunoperoxidase, x200)



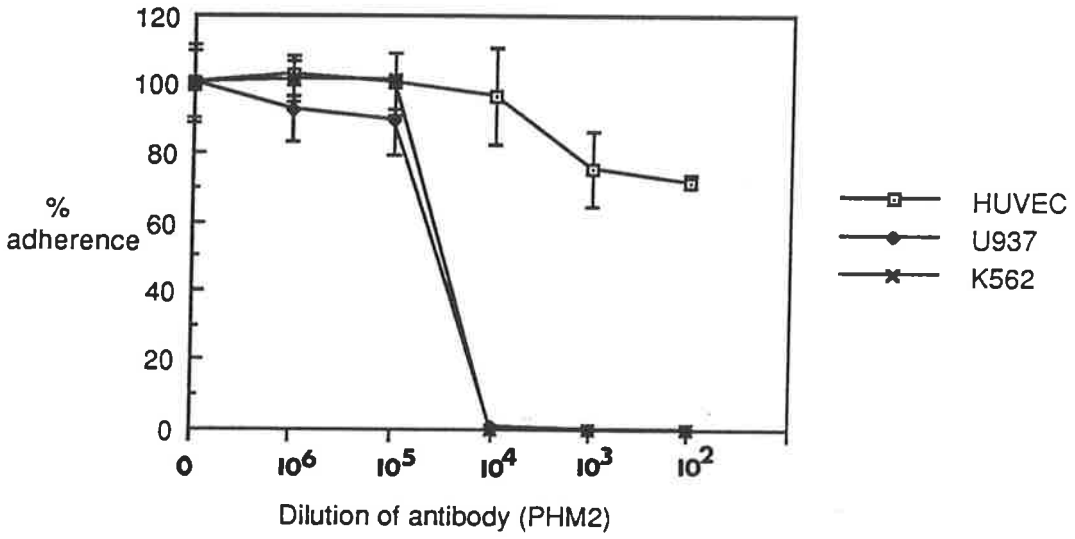
Expression of VLA α_6 chain (GoH3) in the normal human spleen (indirect immunoperoxidase, x200)



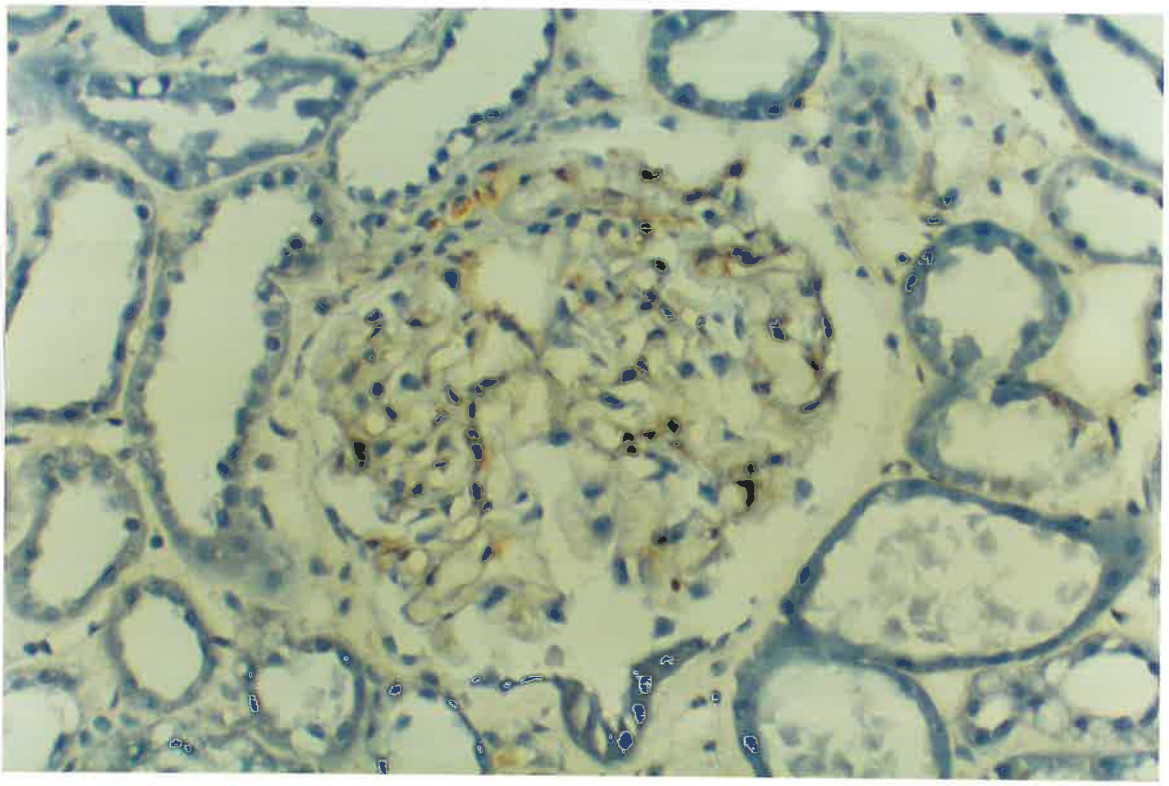
Expression of integrin β_4 chain (S3-41) in normal human spleen (indirect immunoperoxidase, x200)



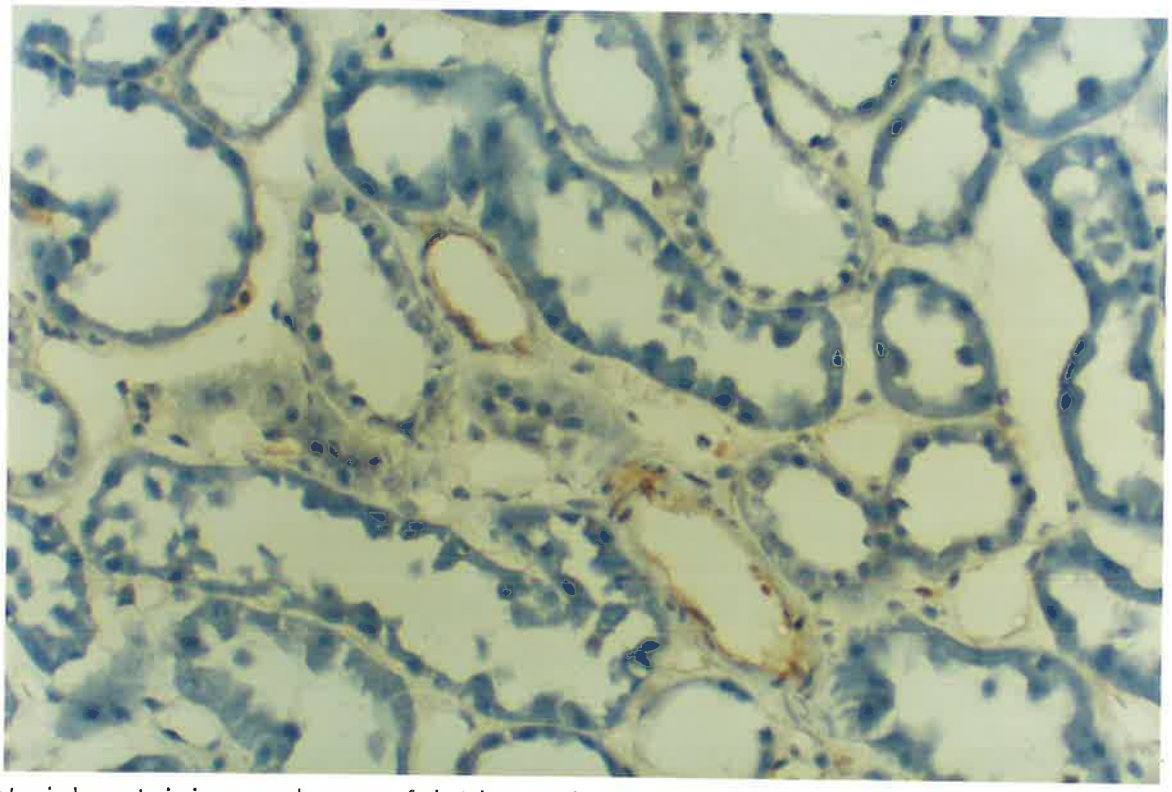
Effects of RMAC11 on adhesion of cultured HUVEC to ECM molecules. The number of cells remaining adherent in the presence of saturating concentrations of RMAC11 is expressed as a percentage of the number of cells in the wells containing the control antibody W6/32 (see materials and methods).



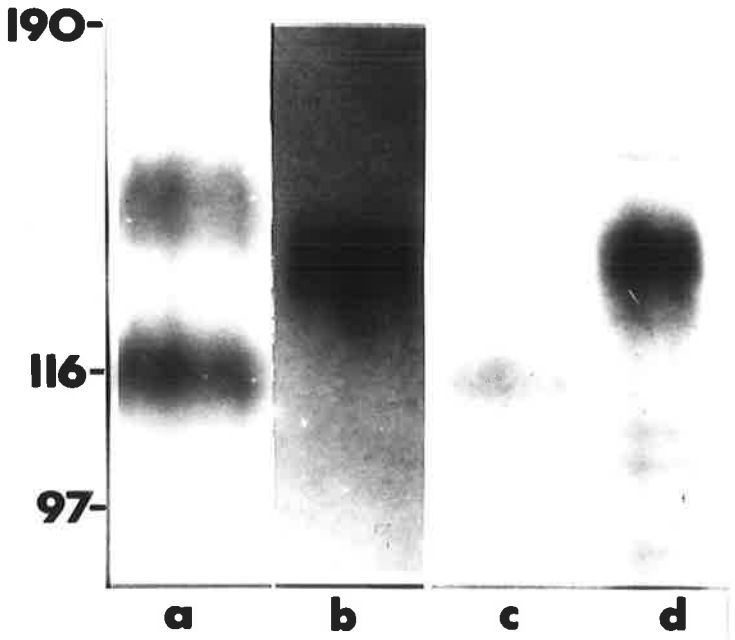
The effect of serial dilutions of PHM2 on the adhesion of HUVEC, U937, and K562 to fibronectin. The number of cells remaining adherent is expressed as a percentage of the number of cells in the wells containing the same dilution of the control antibody W6/32 (see materials and methods)



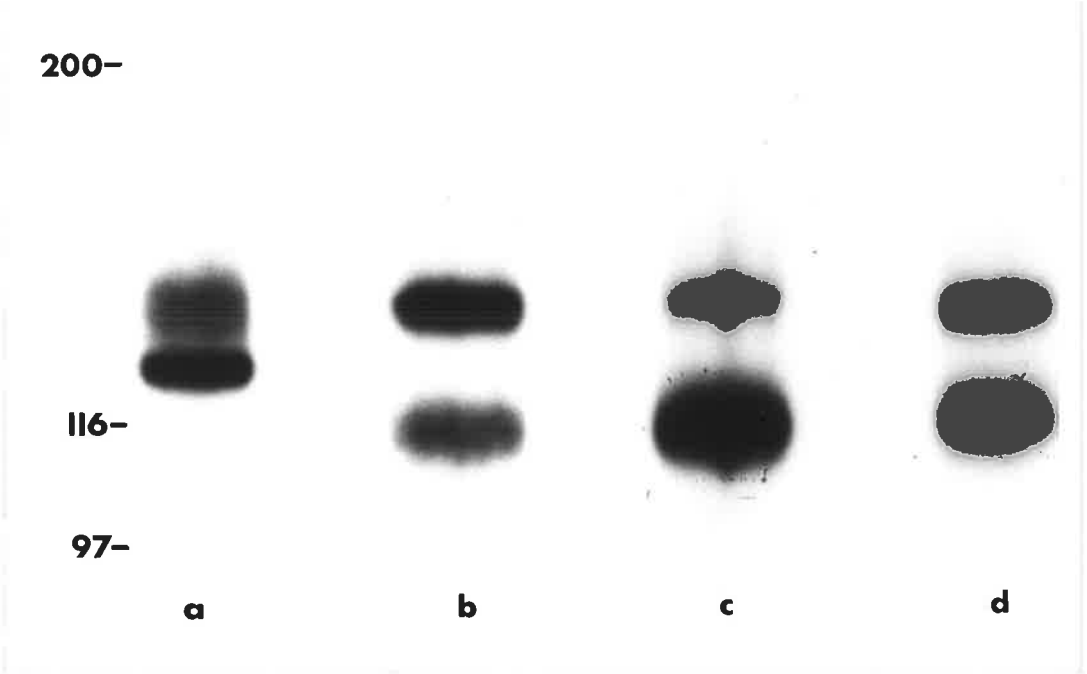
Glomerular staining in a normal kidney with PHM2 (indirect immunoperoxidase, x200)



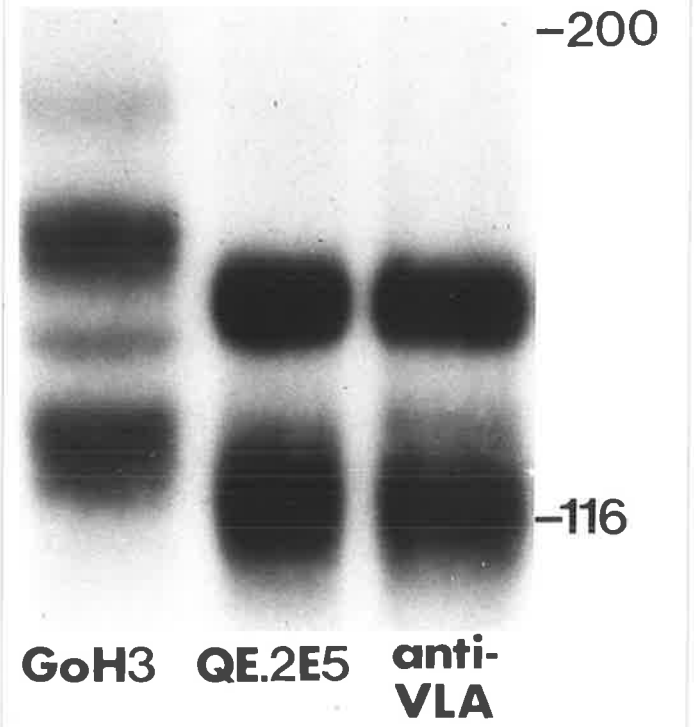
Arteriolar staining and some faint intertubular staining in a normal kidney with PHM2 (indirect immunoperoxidase, x200)



Comparative immunoprecipitation of HUVEC lysate with PHM2 and the anti-VLA-5 antibody B1E5: (a) PHM2 non-reduced (b) PHM2 reduced (c) B1E5 non-reduced (d) B1E5 reduced (5% gel)



Immunoprecipitation from platelet lysate: (a) GoH3 reduced (b) GoH3 non-reduced (c) QE.2E5 non-reduced (d) A-1A5 non-reduced



Immunoprecipitation from HUVEC lysate: (1) GoH3 non-reduced, (2) QE.2E5 non-reduced, (3) goat 172 non-reduced (5% gel)

SECTION 4:
LEUCOCYTE-ENDOTHELIAL INTERACTIONS

4.1 Literature Review

Introduction

The adhesion of leucocytes to the endothelium is a fundamental prerequisite for the emigration of leucocytes into the extravascular tissue. Following this adhesion, the leucocytes slide in between the endothelial cells and pass through a gap they create in the basement membrane by producing matrix-degrading enzymes (Naparstek et al, 1984, Schoefl et al, 1972, Pawlowski et al, 1988). The defect is then rapidly repaired by the overlying endothelial cells. This process has been studied in a model of the venular vessel wall constructed by culturing HUVEC on a collagen matrix (Huber and Weiss, 1989).

Small numbers of leucocytes normally emigrate from the circulation as a part of the physiological trafficking of cells, but they leave it in large numbers in response to inflammation. Lymphocytes are the cells most important in the initiation of alloimmunity, and so most of the following discussion concerns their interactions with the endothelium. This chapter focusses on the receptors for cell-cell interactions, particularly those involved in leucocyte adhesion to the endothelium. The first part of the literature review concentrates on a group of molecules called the "homing receptors", which guide lymphocytes to specific receptors on specialised endothelial cells that line vessels known as high endothelial venules (HEV). These interactions are stabilised by another group of adhesion molecules known as the leucocyte adhesion molecules, which belong to the β_2 integrin family. The wide-ranging roles of this family of adhesion molecules in cell-cell adhesion in the immune system will then be discussed, along with the complementary functions of the receptor-ligand pair called CD2 and LFA-3, which are both members of the immunoglobulin superfamily. Finally, the endothelial receptors for these leucocyte adhesion molecules will be reviewed, with particular emphasis on molecules that appear or are upregulated on endothelial cells after they are activated.

Physiological trafficking of leucocytes

Under normal circumstances, approximately one tenth of the blood neutrophil pool leaves the circulation each hour, although part of this may be in response to subclinical episodes of infection. Monocytes leave the circulation at a lower rate, but do so to become long-lived tissue macrophages (reviewed by Harlan, 1985a). The preferred site of emigration of neutrophils

and monocytes appears to be the postcapillary venule, which is the site of the first major decrease in vessel wall shear stress.

Lymphocytes continually recirculate from the blood to the lymphoid system as they provide immunological surveillance. This enables the lymphocytes, particularly the long-lived memory cells which have been previously exposed to antigen, to gain access to sites of antigenic stimulation. The lymphocytes extravasate in lymphoid tissue at specialised post-capillary vascular sites known as high endothelial venules (HEV) (reviewed: Duijvestijn and Hamann, 1989, Woodruff et al, 1987, Berg et al, 1989). All secondary lymphoid tissues apart from the spleen possess HEV. The endothelial cells that line these vessels have a typical cuboidal, plump appearance (hence "high") (Kraal et al, 1987), in contrast to the usual flattened endothelial cells in other parts of the circulation. This "high" appearance and their specialised function disappears following lymphocyte depletion or blockage of antigen supply from the afferent lymph, but increases rapidly after antigen stimulation. This suggests that factors related to the antigenic stimulation and lymphocyte passage are necessary for maintenance of the characteristic morphology and function of HEV (Duijvestijn and Hamann, 1989). Under normal circumstances, other leucocytes such as neutrophils do not migrate through these HEV in significant numbers (Woodruff et al, 1987).

The HEV develop during the neonatal period in lymph nodes, tonsil, and gut-associated (Peyer's patch and appendix) and bronchial-associated lymphoid tissue. They are found in the cortex and interfollicular regions, but not in the germinal centres nor medulla (Woodruff et al, 1987). Their lining endothelial cells have abundant cytoplasm, large pale nuclei, and a dense nucleolus. They appear to be metabolically active, as they have numerous polyribosomes and a large amount of rough endoplasmic reticulum (Kraal et al, 1987), and take up a large amount of sulphate, which is incorporated into a sulphated glycolipid (Andrews et al, 1982, Andrews et al, 1983). The emigration of the lymphocytes out of the HEV occurs between and not through the cells (Schoefl et al, 1972).

Lymphocyte interactions with the HEV have been extensively studied in vitro using the Stampfer-Woodruff assay (Stampfer et al, 1976). Lymphocytes are overlaid onto frozen sections (fixed or unfixed) of lymphoid tissue, and about 85% of the cells that remain adherent following washing are bound to the HEV, which make up only about 1 to 2% of the surface area of the section. Some studies have been performed with cultured high endothelial cells derived from rat lymph nodes (Ager, 1987, Ager and Mistry, 1988, Ise et al,

1988), or using short-term in vivo homing studies in mice (Stevens et al, 1982). The latter studies have shown that T lymphocytes preferentially distribute to peripheral lymph nodes by binding selectively to their HEV, and B lymphocytes have a preference for gut-associated lymphoid tissue and their HEV (Stevens et al, 1982, Pals et al, 1986). HEV-binding ability is a property of mature lymphocytes rather than thymocytes or bone marrow cells, which are sessile in vivo (Jalkanen and Butcher, 1985). In addition, mouse lymphoma lines have been identified which bind with almost absolute specificity to HEV of peripheral lymph nodes or of Peyer's patch (Butcher et al, 1980). The selective binding of lymphocytes to HEV, and the preference of certain lymphoid cell populations for specific HEV, is now known to be mediated by specialised lymphocyte cell surface structures, which will be discussed later.

Leucocyte extravasation during inflammation

Neutrophils are the predominant infiltrating cells during the early phase of an evolving inflammatory response, but they are gradually replaced by monocytes/macrophages and lymphocytes in the later phases. Increased neutrophil adhesion to the endothelium is specifically confined to the vessel intima immediately adjacent to the acute inflammation, and it is then followed by diapedesis and extravascular migration. Local factors dictate that the neutrophils adhere only at the site of inflammation, as the endothelium in uninvolved areas remains non-adherent (Harlan, 1985a). Chemotactic factors elaborated during the acute inflammation, particularly in bacterial infections, contribute to the attraction of neutrophils to that particular site, but these will not be discussed further here. Considerable evidence exists that the endothelium adjacent to an inflammatory focus undergoes alterations that both make it more adherent and facilitates subsequent leucocyte extravasation. Significantly, the neutrophil adhesion to the endothelium that follows stimulation of the neutrophils alone (eg after systemic complement activation [O'Flaherty et al, 1978], or during the granulocytopenia of haemodialysis [Arnaout et al, 1985]), is transient and not followed by neutrophil emigration from the blood, suggesting that endothelial activation is necessary for extravasation.

The mechanisms of monocyte emigration into an inflammatory focus are similar to those for neutrophils, and again endothelial alterations are particularly significant. In both cases the endothelial cells are induced to

express specific receptors for the leucocytes. These changes will be discussed in more detail later in this chapter.

Lymphocytes have a less prominent role in the typical acute inflammatory response which is characterised by neutrophil infiltration eg that which occurs in response to bacterial infection. However, lymphocytes are the major cells involved in the initiation of allograft rejection (apart from the now rare antibody-mediated hyperacute rejection), chronic inflammatory responses (eg rheumatoid arthritis), and cell-mediated hypersensitivity. Neutrophils only become prominent in the later stages of these reactions when tissue necrosis has occurred. Experimental evidence has shown that alterations in the endothelium at these sites are central events that mediate lymphocyte adhesion and diapedesis. The precise changes are distinct from those that occur during neutrophil and monocyte extravasation, and strongly resemble the interactions between lymphocytes and HEV during normal lymphocyte recirculation. The endothelial cell alterations that facilitate this lymphocyte extravasation are the central theme of this section.

In all types of inflammation that have been studied, the infiltrating leucocytes leave the circulation by traversing post-capillary venules (Dvorak et al, 1986). In acute inflammation, these vessels retain their usual flat appearance, but in various examples of chronic inflammation they develop HEV-like morphology (Dvorak et al, 1986). This suggests that "high" endothelium develops from normally flat endothelium in situations of persistent immune reactivity, analogous to the antigen-stimulated lymphoid tissue.

For example, bulging endothelial cells reminiscent of HEV develop in the peroxidase arthritis model of the immune inflammatory response to a persistent local antigen (horseradish peroxidase) (Graham and Shannon, 1972). The height of the endothelial cells in rheumatoid synovial vasculature is positively correlated with the number and percent of perivascular lymphocytes (Iguchi and Ziff, 1986), and these HEV-like vessels are particularly adhesive for lymphocytes (Oppenheimer-Marks and Ziff, 1986). A specific endothelial cell recognition system that mediates lymphocyte emigration into inflamed synovial tissue has been characterised (Jalkanen et al, 1986b). It is distinct from the recognition system for peripheral lymph node and Peyer's patch HEV. HEV-like venules occur in the lymphocyte aggregates of a variety of pathological conditions (Freemont, 1983), including foreign body reactions, ulcerative colitis, peptic ulceration, rheumatoid synovitis, chronic cholecystitis, chronic pyelonephritis, myositis, and Hashimoto's

thyroiditis. The number and size of the vessels varies with lymphocyte density rather than the underlying condition. HEV-like changes also occur in the post-capillary venules that are surrounded by lymphocytic infiltration in BCG-induced granulomata in rat skin (Freemont and Ford, 1985). It appears that any lymphocytic collection in chronic inflammatory disorders can be associated with HEV-like vessels.

The development of HEV-like vessels at sites of chronic inflammation suggests that the molecular mechanisms of lymphocyte adhesion to and extravasation through this activated endothelium might be similar to those that operate in the HEV of lymphoid tissue.

The microvascular endothelium is a major site of damage during allograft rejection (Dvorak et al, 1979, Bishop et al, 1989b). Sequential examination of the ultrastructural changes during first set rejection of cardiac allografts in the rat shows a severe loss of functional and structural integrity of the microvascular endothelium that precedes the development of extensive damage to the cardiac muscle cells (Forbes et al, 1983). At the onset of the rejection, the extensive mononuclear extravasation is accompanied by the development of "high activated endothelium", similar to the changes observed in models of delayed-type hypersensitivity (Dvorak et al, 1976), skin grafts (Dvorak et al, 1979), and renal allografts (Pederson and Morris, 1970). HEV-like vessels appear in sponge matrix allografts that have acquired alloantigen-reactive T lymphocytes (Bishop et al, 1989), and these vessels bind lymphocytes in ex-vivo studies. Sponge matrix isografts do not develop the same vessel changes. The peritubular capillary endothelium of a renal allograft preferentially binds lymphocytes in much the same way as HEV, and this is the most likely site of the influx of leucocytes into the graft during rejection (Renkonen et al, 1989b). These endothelial cells show signs of marked activation during allograft rejection (Renkonen et al, 1990).

It is postulated that these microvascular changes are secondary to cytokines released by the infiltrating mononuclear cells. Lymphocytes (particularly CD8-positive cytotoxic T lymphocytes) induce marked morphological changes in cultured allogeneic endothelial cells and induce the expression of MHC class II antigens (Bender et al, 1989). The intradermal injection of crude lymphokine, containing both tumour necrosis factor (TNF) and interferon activities, induces vascular changes similar to those in a delayed-type hypersensitivity skin reaction (Dumonde et al, 1982). TNF and γ-IF injected into baboon skin induce endothelial cell hypertrophy, increased vascular permeability, and a progressive leucocyte infiltration that resembles a

delayed hypersensitivity reaction (Munro et al, 1989). Patients treated with IL-2 immunotherapy for metastatic malignancy often develop a vascular leak syndrome, and skin biopsies show endothelial cells that appear to be activated and "leaky" (Cotran et al, 1987). The proposed mechanism for this endothelial activation is that IL-2 induces the production of other cytokines by mononuclear cells, which then mediate these changes. IL-2 itself has no direct effect on endothelial cells in culture.

Cultured HUVEC treated with IL-1 or TNF plus γ-IF change from their typical polygonal epithelial-like shape to plump, retracting cells with prominent intercellular gaps (Stolpen et al, 1986). In another study, stimulation of cultured HUVEC with TNF or lymphotoxin (LT) resulted in increased adhesiveness for lymphocytes, increased metabolism (measured by RNA and protein synthesis), and increased cell volume (Cavender et al, 1989). The changes are reversible upon removal of the cytokines, and may represent the in vitro equivalent of the development of HEV-like vessels in inflammatory foci.

It has been suggested that the role of γ-IF in these studies is to convert the changes of acute inflammation into those of chronic (or immune) inflammation (Pober, 1988). Antibodies against γ-IF inhibit the development of delayed-type hypersensitivity inflammatory changes in rat skin (Issekutz et al, 1988).

IL-1, TNF, lymphotoxin (LT), and γ-IF, singly or in combination, have other effects on endothelial cells in vitro, including the induction of "activation antigens" and increased endothelial adhesiveness, which will be discussed later in this section. These effects and the morphological changes seem to constitute the in vitro equivalent of the development of HEV-like vessels in vivo.

Lymphocyte adhesion to HEV and HEV-like vessels, and their subsequent extravasation, is mediated by specific receptors on the surface of the lymphocytes binding to their ligands on the endothelial cells. The lymphocyte molecules that mediate binding to HEV in lymphoid tissue are called "homing receptors" because they guide the homing of the lymphocytes to specific sites in the lymphoid system.

Lymphocyte Homing Receptors

(1) Peripheral lymph node HEV receptor

The most extensively studied homing receptor mediates the adhesion of lymphocytes to peripheral lymph node HEV in mice. It was originally defined

by the rat monoclonal antibody MEL-14 (Gallatin et al, 1983), which binds specifically to lymphocytes and lymphoid cell lines which adhere to peripheral lymph node HEV. MEL-14 almost completely inhibits this adhesion (in vitro and in vivo), without having any effect on those cells which adhere to Peyer's patch HEV. It inhibits the development of lymphadenopathy associated with autoimmunity in MRL-lpr/lpr mice, probably by interfering with lymphocyte homing to the peripheral lymph nodes (Mountz et al, 1988). Germinal centre lymphoid cells, which are nonmigratory and fail to bind to HEV or migrate normally in lymphoid tissues, do not express the MEL-14 antigen (Reichert et al, 1983). These lymphocytes have lost their ability to home after being activated by antigens. In vitro, lymphocytes rapidly shed MEL-14 after phorbol ester stimulation, and the shed fragment can be detected in the supernatant (Kishimoto et al, 1990).

MEL-14 immunoprecipitates an 80-90kD antigen from lymphocytes which migrates more rapidly under non-reducing conditions on SDS-PAGE, suggesting that it has significant intrachain disulphide bonding. The size also varies slightly according to the cellular source of antigen. Initial studies of the structure of the MEL-14 antigen revealed two amino termini, one of which corresponds to the amino terminus of ubiquitin, an 8.4 kD polypeptide that has been remarkably conserved through evolution (Siegelman et al, 1986).

The receptor recognised by MEL-14 is carbohydrate-binding (Yednock et al, 1987), and the purified antigen interacts in a sugar-inhibitible manner with sites on peripheral lymph node HEV which prevents attachment of lymphocytes (Geoffrey and Rosen, 1989). The MEL-14 antibody binds to a determinant in the carbohydrate-binding (lectin) domain of the receptor (Bowen et al, 1990). Cloning of the receptor has revealed the anticipated lectin domain (Lasky et al, 1989), which is highly homologous to domains found in a diverse series of calcium-dependent animal lectins. The lectin domain is followed by two other protein domains - an epidermal growth factor (EGF)-like domain, and two homologous repeat units preserving the motif of complement regulatory proteins. The additional domains suggest interesting functional possibilities, and this arrangement is also present in three other molecules - endothelial-leucocyte adhesion molecule-1 (ELAM-1), granule membrane protein-140 (GMP-140), and the human equivalent of the MEL-14 receptor (leucocyte adhesion molecule-1, LAM-1). These four molecules make up a recently identified family of adhesion receptors (the "selectins" [Tedder et al, 1990]) that will be discussed in more detail later.

The precise structure of the ligand on endothelial cells for the MEL-14 receptors is unknown, but it presumably has a carbohydrate recognition determinant that binds to the lectin domain. Stoolman et al (1983) found that L-fructose, D-mannose, and the L-fucose-rich sulphated polysaccharide fucoidin specifically inhibit the binding of lymphocytes to HEV. Sialic acid residues appear to be important, as sialidase treatment of lymph node sections removes sialic acid residues and inhibits lymphocyte binding to HEV (Rosen et al, 1985), and sialidase injected intravenously into mice selectively prevents subsequent in vitro attachment of lymphocytes to peripheral lymph node HEV but not Peyer's patch HEV (Rosen et al, 1989). Sialic acid determinants are well placed for recognition function as they are generally found in a terminal, non-reducing position of oligosaccharide chains, and they apparently serve as recognition determinants in a number of microbial-host interactions (Sharon et al, 1989).

Human lymphocytes and lymphoid cell lines possess functional homing receptors for binding to HEV (Navarro et al, 1985, Stoolman and Ebling, 1989b), and a functionally similar carbohydrate-binding receptor mediates selective attachment of human peripheral blood lymphocytes to the HEV of human lymph nodes (Stoolman et al, 1987). The human equivalent of the murine MEL-14 has been identified, both by using the murine MEL-14 receptor cDNA clone to identify the human receptor (Bowen et al, 1989), and by isolating the clone from a human cDNA library (Siegelman and Weissman, 1989, Tedder et al, 1989). It is highly homologous (77%) to the murine receptor, and as expected, contains the lectin domain located at the amino terminus followed by an EGF-like domain and a domain containing two repeats of a complement-binding motif.

The human receptor has been named leucocyte adhesion molecule-1 (LAM-1) (Tedder et al, 1989), and is identical to the previously described pan-leucocyte antigen Leu-8/TQ1 (Camerini et al, 1989), which identifies the suppressor-inducer subset of $CD4^+$ T lymphocytes (Reinherz et al, 1982). A possible explanation for the helper-inducer subset lacking this receptor is that it comprises activated cells which have exited the lymph nodes into the peripheral circulation, and while doing so shed their LAM-1. It is bound to the cell by either a conventional transmembrane segment or by a phospholipid anchor, and the latter may be shed following activation or homing (Camerini et al, 1989). Cell lines which express high levels of LAM-1 mRNA bind to human HEV, while most of those that do not express LAM-1 mRNA do not bind (Tedder et al, 1989). Blood lymphocytes exposed to the phorbol ester PMA

rapidly modulate LAM-1 from the cell surface, and at the same time lose their ability to bind to lymph node HEV (Tedder et al, 1990, Oppenheimer-Marks et al, 1990). Thus the evidence strongly suggests that LAM-1 mediates the same function in humans as the MEL-14 receptor in mice i.e. lymphocyte binding to peripheral node HEV, and that it is lost from the cell surface following activation or extravasation (Hamann et al, 1988b).

The MEL-14 receptor/LAM-1 is also expressed by neutrophils, monocytes, eosinophils, marrow myeloid cells, granulocyte/macrophage colony-forming units, and burst-forming units for erythroid cells (Lewinsohn et al, 1987, Tedder et al, 1990, Griffin et al, 1990). The MEL-14 antibody inhibits the in vitro adhesion of neutrophils to HEV in peripheral lymph nodes, and inhibits the capacity of neutrophils to migrate into sites of acute inflammation in the skin. This shared recognition mechanism between lymphocytes and neutrophils is a little surprising considering the known preferences of lymphocytes for lymphoid tissue (although neutrophils can migrate into inflamed lymphoid tissue), and neutrophils for sites of acute inflammation. One possible explanation is that other receptors contribute to the specificity of sites of leucocyte adhesion and extravasation.

Stimulation of murine neutrophils with chemotactic factors results in a rapid loss of the MEL-14 antigen from the cell surface by shedding (Kishimoto et al, 1989, Jutila et al, 1989b), which is in direct contrast to the increased expression by neutrophils of other adhesion molecules (see later) following stimulation. The loss of MEL-14 antigen is associated with loss of the neutrophil's ability to bind to inflammatory sites in vivo. LAM-1 on human neutrophils, monocytes, and their precursors is also downregulated rapidly following stimulation with GM-CSF, TNF, FMLP, and leukotriene B4 (Griffin et al, 1990). It has been postulated that the MEL-14 antigen (and presumably LAM-1 in humans) mediates the initial interaction beween neutrophils and the vascular endothelium during the early phases of an inflammatory response, and, upon activation of the neutrophils, the other upregulated adhesion molecules "take over" and mediate the subsequent emigration from the vasculature (Jutila et al, 1989a). Clearly, the identity of the unknown ligand for neutrophil MEL-14 antigen on inflamed endothelium is of great interest. Presumably it is expressed only on endothelial cells in inflamed tissue or in HEV (or in a modified form at those sites), as neutrophils do not exhibit the same adhesion for normal endothelium, and their low constitutive binding to HEV is dramatically increased by inflammatory stimuli. Monocytes bind poorly to HEV in uninflamed lymph nodes, but also exhibit a dramatic increase in

adherence following inflammation, which is also inhibited by the MEL-14 antibody (Jutila et al, 1989a).

It is apparent that the MEL-14/LAM-1 homing receptors have more complex roles than just the homing of lymphocytes to HEV in peripheral lymph nodes. A further intriguing possibility is that the shed MEL-14 antigen/LAM-1 may have a modulating role at sites of inflammation by binding to the endothelial ligand and preventing further adhesion of leucocytes.

(2) Peyer's patch HEV receptor

Functional studies identified a distinct lymphocyte receptor for Peyer's patch HEV, and it has been recently characterised with the rat anti-mouse monoclonal antibody R1-2 (Holzmann et al, 1989a), which inhibits the adhesion of murine lymphocytes to Peyer's patch HEV but not to peripheral lymph node HEV. The receptor is called lymphocyte Peyer's patch HEV adhesion molecule-1 (LPAM-1), and is a heterodimer. The estimated weight of the chains is 160 and 130 kD, and R1-2 binds to the larger (α) subunit. LPAM-1 is structurally similar to the ECM receptor VLA-4, and anti-VLA-4 α chain antiserum recognises the α subunit of LPAM-1. In addition, antibodies against VLA-4 have been found to inhibit the binding of human lymphocytes to appendix HEV (Berg et al, 1989).

The amino acid sequences of the two α chains are highly homologous, but the LPAM-1 α chain (α_{4M}) is associated with a novel β chain (β_P) on mouse lymphoma cells that selectively bind to Peyer's patch HEV (Holzmann et al, 1989b). The β_P chain is distinct from the β_1, β_2, and β_3 integrin chains. The α_{4M} chain can also associate with the integrin β_1 chain, and this complex is involved in the adhesion of certain cells to Peyer's patch HEV. Some cells express both the α_{4M}/β_P and the α_{4M}/β_1 complexes.

VLA-4 is also a receptor for a recently described molecule on activated endothelial cells (see later), and it is likely that lymphocyte receptors possessing the LPAM-1/VLA-4 α chain have an important role in the adhesion of lymphocytes to endothelium at inflammatory sites.

(3) Hermes receptor (CD44)

The monoclonal antibody Hermes-1 identifies a 90 kD lymphocyte surface glycoprotein that is involved in endothelial cell recognition and lymphocyte trafficking in man (Jalkanen et al, 1986a). The Hermes antigen is selectively

expressed by lymphoid cells that recognise and bind to HEV, but the Hermes-1 antibody does not block the adhesion. The Hermes-3 antibody was produced by immunisation with Hermes-1 antigen isolated from a cell line that specifically binds to mucosal HEV (Jalkanen et al, 1987). This antibody selectively blocks lymphocyte adhesion to mucosal lymphoid tissue HEV (ie Peyer's patch, appendix) in humans. A distinct recognition system involving the Hermes antigen also exists on lymphocytes for the HEV-like vessels in inflamed synovium from patients with rheumatoid arthritis (Jalkanen et al, 1986b). Polyclonal antiserum produced against isolated Hermes antigen blocks lymphocyte adhesion to HEV in peripheral lymph nodes, mucosal tissue, and inflamed synovium, which implicates the Hermes molecules in all three pathways of lymphocyte trafficking (Jalkanen et al, 1987). These findings suggest that the Hermes antigen actually comprises a family of related but functionally distinct receptors for HEV, or that it associates both physically and functionally with such receptors.

Sequential immunoprecipitation studies show that the Hermes-1, Hermes-3, and polyclonal antiserum immunoprecipitate the same antigen (Jalkanen et al, 1987), and the Hermes-1 antibody immunoprecipitates physicochemically identical species from peripheral blood lymphocytes and from cell lines that specifically adhere to mucosal or peripheral lymph node HEV (Jalkanen et al, 1988).

The Hermes molecule has now been cloned (Stamenkovic et al, 1989, Goldstein et al, 1989) and designated CD44. It is synthesised as a 37 kD polypeptide backbone that is either glycosylated to the usual 85 to 95 kD form, or additionally modified by covalent linkage to chondroitin sulphate to a 180 to 200 kD form (Jalkanen et al, 1988, Idzerda et al, 1989). A distal region of the amino acid sequence shows striking homology to tandemly repeated domains of the chicken and rat cartilage link and proteoglycan core proteins, but no homology to the MEL-14 antigen or integrin families (Goldstein et al, 1989). This domain is highly conserved between humans and mice (Zhou et al, 1989). The cartilage link proteins stablilise large aggregates of cartilage proteoglycan core proteins by binding to proteoglycan monomers or to hyaluronic acid, and can also bind to native collagen (Haynes et al, 1989). The extracellular matrix receptor III (ECMR III), that is identical to CD44, was originally isolated on collagen-affinity columns (Carter and Wayner, 1988), and CD44 has recently been shown to be the principal cell surface receptor for hyaluronate (Aruffo et al, 1990, Miyake et al, 1990). This CD44/hyaluronate interaction mediates the adhesion between B lymphocyte hybridoma cells and

a bone marrow stromal cell line, and may account for a number of the multiple functions that have been attributed to CD44.

CD44 cDNAs from different lymphoid cell lines have the same major sequence (Stoolman et al, 1989a). However, the CD44 from different cell types have distinct electrophoretic mobility, presumably due to differential glycosylation (Kansas et al, 1989). This suggests that tissue-specific homing that depends on CD44 is governed by isoforms that differ in carbohydrate residues rather than primary sequence, or that CD44 plays a role in homing by virtue of physical association with distinct molecules that confer the tissue specificity (Berg et al, 1989). One group produced anti-CD44 monoclonal antibodies by immunising with purified lymphocyte function-associated antigen-1 (LFA-1) (see later), which suggests that they are closely associated on the cell surface and were co-purified, or that they share epitopes (Pals et al, 1989b). It is likely that the organ-specific component of lymphocyte-HEV adhesion is the product of combinations of multiple adhesion molecules rather than a single receptor (Holzmann et al, 1989a).

The CD44 antigen is identical to a widely distributed multifunctional cell surface molecule that has been described in a number of studies (reviewed by Haynes et al, 1989). The various names include Pgp-1, ECMR III, and p80 in humans, and Ly24 and Pgp-1 in mice (Gallatin et al, 1989, Picker et al, 1989). It is expressed by lymphoid, myeloid, erythrocyte, epithelial, endothelial, glial, and fibroblast populations (Carter and Wayner, 1988). Memory T lymphocytes, which have been previously activated by antigen, stably acquire CD44 (Budd et al, 1987). They also express increased levels of other adhesion molecules (LFA-1, CD2, LFA-3, CD29), which may provide them with a selective advantage when they again encounter the antigen that provided the initial stimulation (Sanders et al, 1988a, Sanders et al, 1988b).

CD44 is involved in cell-cell interactions other than lymphocyte adhesion to HEV. The expression of CD44 by lymphomas correlates with their metastatic potential (Pals et al, 1989a), and mouse L cells transfected with CD44 cDNA exhibit a new self adhesive phenotype, forming large aggregates in suspension (St. John et al, 1990). Antibodies against CD44 inhibit erythrocyte adhesion to human T lymphocytes by binding to the CD44 on erythrocytes (Hale et al, 1989b, Shimizu et al, 1989). The anti-CD44 antibodies block erythrocyte adhesion by interfering with the CD2/LFA-3 interaction between erythrocytes and T lymphocytes (Hale et al, 1989b). CD44 on erythrocytes is regulated by the In(Lu) dominant inhibitor gene and expresses the In^a and In^b blood group antigens.

CD44 also has a role in cell activation. Anti-CD44 monoclonal antibodies augment T lymphocyte proliferation induced by CD3- and CD2-receptor-mediated activation (Shimizu et al, 1989, Huet et al, 1989, Denning et al, 1990). Huet et al postulated that the binding of T lymphocytes to CD44 on endothelial cells provides an accessory signal in T lymphocyte activation. Denning et al also found that anti-CD44 antibodies enhance monocyte IL-1 release, adhesion of T lymphocytes and monocytes in anti-CD2 antibody-stimulated cultures, and T lymphocyte IL-2 production in response to anti-CD2 antibodies. In contrast, soluble CD44 found in the serum may have a regulatory role by competing with cell-bound CD44 for its natural ligand (Lucas et al, 1989).

Leucocyte Adhesion Molecules

(1) Introduction

The β_2 integrin family consists of three heterodimers that share a common β chain non-covalently linked to distinct α chains. They are only expressed by leucocytes. One member of the family, lymphocyte function-associated antigen-1 (LFA-1), has an accessory role in lymphocyte homing to lymphoid tissue. Antibodies against LFA-1 inhibit lymphocyte adhesion to HEV by 50 to 60%, but this is not tissue specific, and cells that lack LFA-1 adhere normally to HEV (Hamann et al, 1988a, Pals et al, 1988, Berg et al, 1989). The interpretation of this finding is that LFA-1 cooperates with the lymphocyte homing receptors in the adhesion to HEV, but that the latter receptors confer tissue specificity.

The β_2 integrin family has a much wider role in general immune functions than the lymphocyte homing receptors, including participation in the interactions of leucocytes with normal endothelium. Adhesion between leucocytes and other cells is a requirement of virtually every step during the immune response. Some examples are: the leucocyte adhesion to endothelial cells at sites of inflammation and in HEV; activation of T lymphocytes by interaction with antigen-presenting cells; interactions between the regulatory and effector T lymphocytes; help provided by T lymphocytes for B lymphocytes; and the killing of target cells by cytotoxic T lymphocytes, natural killer cells, neutrophils, and monocytes. Most of these interactions can be fundamentally divided into two phases. The first is the antigen-specific

recognition step (eg between the T cell receptor (TCR) and the MHC complex on the antigen-presenting cell), and the second is non-antigen-specific adhesion mediated by adhesion molecules on each of the cells, which stabilises the interaction once specific recognition has occurred.

The interaction between cytotoxic T lymphocytes (CTL) and their targets has been extensively studied (reviewed Martz, 1987). Its specificity is determined by the TCR and the target cell MHC, and antibodies against the TCR or its associated CD3 complex will inhibit cytolysis. However, this inhibition is not due to blockage of adhesion between the cells as they continue to form conjugates in the presence of these antibodies (Spits et al, 1986). The TCR/MHC interaction is insufficient by itself to maintain apposition between the cells (except in the case of some CTL clones), so stabilisation depends on an ancillary non-antigen specific adhesion mechanism. Blocking this adhesion inhibits conjugate formation, TCR/MHC interaction, and the cytolysis. Nonspecific adhesion of the CTL to their target cells precedes the specific alloantigen recognition (Spits et al, 1986).

Macrophages frequently form weak, transient interactions with other cells, but strong, prolonged adhesion only develops when activated macrophages bind to specific target neoplastic cells (Somers et al, 1986). They postulated that cells can non-specifically adhere to each other via adhesion molecules, and if specific cell recognition follows, the effector cell becomes activated which in turn results in strengthening of the adhesion. The stabilisation of the binding requires time and metabolic activity within the effector cell, and appears to be due to increased avidity of the effector cell adhesion molecules for their ligands on the target cells. This could be due to increased cell surface expression, clustering, and/or an alteration in their physical state that increases their affinity.

The adhesion molecules that mediate CTL/target cell adhesion were identified by screening for monoclonal antibodies that block CTL-mediated killing. In this way, lymphocyte function-associated antigen-1 (LFA-1) was identified in mice (Davignon et al, 1981b) and then in humans (Sanchez-Madrid et al, 1982). Two other molecules that stabilise intercellular adhesion were also identified in the latter study. These molecules (LFA-2, now referred to as CD2, and LFA-3) are a receptor-ligand pair, and will be discussed later. They are not structurally related to LFA-1.

Subsequent studies have shown that antibodies against LFA-1 block virtually every immune response that requires T lymphocyte adhesion (Davignon et al, 1981a, Beatty et al, 1983, Hildreth et al, 1983, Hildreth et al,

1985, Krensky et al, 1983, Springer et al, 1987, Mazerolles et al, 1988). They block CTL-mediated killing by preventing the Mg^{++}-dependent adhesion rather than the Ca^{++}-dependent lethal hit delivery. They also inhibit natural killer cell-mediated and lymphokine-activated killer (LAK)-mediated cytolysis (Mentzer et al, 1986b, Axberg et al, 1987, Martz, 1987, Timonen et al, 1988).

Human LFA-1 is a heterodimer consisting of a 177 kD α subunit associated with the 95 kD β_2 chain (Sanchez-Madrid et al, 1982). The other β_2 integrins, Mac-1 (or complement receptor type 3, CR3) and p150,95 (Sanchez-Madrid et al, 1983), have 165 and 150 kD α subunits respectively. The β_2 subunit has been designated CD18, and the α subunits of LFA-1, Mac-1, and p150,95 designated CD11a, b, and c, respectively. The α subunits are all encoded within a small area of chromosome 16, which defines a gene cluster, whereas the β_2 subunit is encoded on chromosome 21 (Corbi et al, 1988a).

The α and β chains have significant homology to other members of the integrin superfamily (Hynes, 1987). The β_2 chain is approximately 45% homologous to the chicken β_1 subunit (Kishimoto et al, 1987a, Low et al, 1987). The LFA-1 α subunit has 36% homology with the Mac-1 and p150,95 α subunits, and 28% identity to other integrin α subunits (Larson et al, 1989). The Mac-1 α subunit is 63% identical to the α subunit of p150,95, and 25% identical to other integrin α subunits (Corbi et al, 1988b, Hickstein et al, 1989). The β_2 integrins are all dependent on divalent cations for their functions, and the α subunits contain highly conserved divalent cation-binding sequences.

(2) LFA-1

LFA-1 is the only β_2 integrin expressed by lymphocytes. It is also found on monocytes, neutrophils, large granular lymphocytes (which include natural killer cells), eosinophils, basophils, and about 50% of bone marrow cells (Krensky et al, 1983, de Boer and Roos, 1986). It is not found on non-haematopoietic cells, including endothelium (Mentzer et al, 1986a). Lymphocytes and neutrophils have no significant intracellular stores of LFA-1, and its surface numbers do not change after stimulation with chemoattractants (Anderson et al, 1987, Rothlein and Springer, 1986a, Pichyangkul et al, 1988). However, activated and memory T lymphocytes express increased LFA-1 (Sanders et al, 1988a, Sanders et al, 1988b). In contrast, monocytes rapidly upregulate LFA-1 upon stimulation, but whether this comes from an intracellular pool is unknown (Miller et al, 1987b).

The α subunit has a polypeptide backbone of 149 kD and is synthesised as a precursor of 165 kD by addition of N-linked carbohydrates, followed by conversion to the mature form (Miller and Springer, 1987a).

In experimental studies the functions of LFA-1 on neutrophils and monocytes are best dissected out by the use of antibodies directed against the α subunit, as anti-β_2 antibodies also affect Mac-1 and p150,95. However, antibodies against either subunit can be used with lymphocytes as they only express LFA-1.

Antibodies against LFA-1 inhibit multiple T lymphocyte adhesion-dependent functions. They also inhibit adhesion of other leucocytes, including macrophage adhesion to tumour cell targets (Strassman et al, 1986) and the spontaneous and phorbol ester-stimulated homotypic adhesion of B lymphocytes and B lymphoblastoid cells (Patarroyo et al, 1983b, Patarroyo et al, 1985, Mentzer et al, 1985, Rothlein and Springer, 1986a). The increased adhesion after phorbol ester stimulation is due to conversion of the LFA-1 to a high avidity state for its ligand (Dustin and Springer, 1989), which may be as a result of a conformational change. This high avidity state is transient, peaks after 5 to 10 minutes, and is followed by return to the original low avidity state by 30 minutes to 2 hours. An antibody against a particular epitope on the α chain also stimulates homotypic adhesion with similar kinetics as the phorbol ester, again possibly by inducing a conformational change (Keizer et al, 1988). The activation of T lymphocytes through the TCR (by anti-TCR or anti-CD3 antibodies) or through CD2, or of antigen-presenting cells through their MHC class II molecules, also stimulate homotypic adhesion via the same protein kinase C pathway as phorbol esters (Dustin and Springer, 1989, van Kooyk et al, 1989, Mourad et al, 1990). Phorbol esters induce phosphorylation of the β_2 chain but have no effect on the α chain, which is constitutively phosphorylated (as are CD11b and CD11c) (Chatila and Geha, 1988, Buyon et al, 1990). The phosphorylation may alter the interaction between the cytoplasmic portion of the β subunit and the cytoskeleton, resulting in the predicted conformational change. The increased adhesion induced by phorbol esters is sensitive to cytochalasin B, which interferes with actin in the cytoskeleton (Martz, 1987).

These studies suggest that engagement of the TCR to the target MHC-antigen complex triggers an adhesion amplification mechanism, which is necessary to stabilise the conjugation formation. The message that increases the avidity of LFA-1 is transmitted by intracellular signalling pathways such as via protein kinase C. The increased avidity is transient, which allows the cells

to disengage once the appropriate interaction has been completed (eg delivery of the "lethal hit" by CTL).

This cycle of adhesion and de-adhesion may operate in a similar way during cellular migration through the extracellular matrix, and T lymphocyte chemotaxis in response to IL-2 or lymphocyte chemotactic factor is inhibited by anti-CD11a and anti-CD18 antibodies (van Epps et al, 1989).

There is also evidence that the engagement of LFA-1 with its ligand on the target cell transmits signals to the LFA-1-bearing cell. Antibody to LFA-1 induces changes in murine B lymphocytes that mimics the activating effects of B cell stimulatory factor-1 (BSF-1, IL-4) (Mishra et al, 1986). Another study found that 5 out of 6 anti-CD18 antibodies inhibited T lymphocyte proliferation induced by immobilised anti-CD3 antibodies, whereas all 9 anti-CD11a antibodies enhanced proliferation to varying degrees (van Noesel et al, 1988). Cross-linking LFA-1 on human T cell clones enhances anti-CD3-induced calcium flux, IL-2 production, and cell proliferation (Wacholtz et al, 1989). Cross-linking with anti-CD11a antibodies by itself induces phosphoinositide hydrolysis and a rise in intracellular calcium (Pardi et al, 1989). Immobilised antibodies against CD11a or CD18 on monocytes induce their expression of cell-associated IL-1 without release of soluble IL-1 into the medium (Couturier et al, 1990). Finally, the interaction between LFA-1 and its ligand (ICAM-1 - see later) provides a potent costimulatory signal with immobilised anti-CD3 antibodies against resting T lymphocytes (van Seventer et al, 1990).

In summary, it appears that activation of the cell increases adhesiveness by altering LFA-1, and that interaction of LFA-1 with its ligand stimulates intracellular signals that influence the cell's behaviour. This suggests the possibility that engagement of adhesion molecules with their ligands (which may be on other cells or part of the ECM) could generate signals that regulate cell growth, function, and differentiation.

(3) Mac-1

The second member of the β_2 integrin family was originally identified in mice with the Mac-1 monoclonal antibody (Springer et al, 1979). Other antibodies against this molecule include Mo1 (Todd et al, 1981, Todd et al, 1982) ,OKM1, and OKM10 (Wright et al, 1983). Antibodies against Mac-1 block the adhesion of myeloid cells to C3bi-coated particles (Beller et al, 1982), demonstrating that it is identical to the complement receptor for C3bi (CR3). The Mac-1 extracellular sequence contains a domain of 187 amino

acids (which is also found in p150,95) that is homologous to the A domains of von Willebrand factor, which in turn are homologous to regions of the C3-binding proteins factors B and C2 (Corbi et al, 1988b). This region is absent from other integrins and is a potential binding site for C3bi. Mac-1 binds to a region of C3bi that contains the RGD sequence, but a hexapeptide containing RGD does not inhibit the interaction, suggesting that other residues contribute to the binding (Wright et al, 1987).

Mac-1 is expressed by neutrophils, monocytes, macrophages, large granular lymphocytes, eosinophils, and basophils, and mediates neutrophil adhesion to endothelial cells and a variety of substrates (Anderson et al, 1986, Wallis et al, 1986). Adhesion functions of neutrophils are most inhibited by anti-Mac-1 antibodies, followed by anti-p150,95 and anti-LFA-1 antibodies. Antibodies against Mac-1 also inhibit neutrophil aggregation (Schwartz et al, 1985) and chemotaxis, phagocytosis by neutrophils and monocytes, and cell lysis by neutrophils, monocytes, eosinophils, and large granular lymphocytes (Kuypers and Roos, 1989). In addition, the large release of hydrogen peroxide by neutrophils stimulated with TNF while they are adherent to ECM proteins is also inhibited by antibodies against CD18 (Nathan et al, 1989). The differentiation of monocytes into tissue macrophages is accompanied by a considerable decrease in Mac-1 expression (Hogg et al, 1986).

Mac-1 is stored in neutrophils and monocytes on the membranes of intracellular peroxidase-negative granules, and rapidly mobilised to increase surface expression after inflammatory stimuli (Todd et al, 1984, Miller et al, 1987b, Bainton et al, 1987, Pichyangkul et al, 1988). This increased expression parallels but does not totally account for the increased adhesiveness of the cells after stimulation. Mac-1 is activated by such stimuli and it becomes more avid for its ligand, analogous to LFA-1 (Philips et al, 1988, Vedder and Harlan, 1988a). The effect of phorbol esters is biphasic. Stimulation for 10 minutes enhances receptor activity, but it decreases to levels below resting cells after a further 60 minutes of stimulation (Wright et al, 1986a). This pattern of alteration does not correlate with the number of cell surface receptors, but is consistent with qualitative changes in receptor adhesiveness. The clustering of receptors that follows phorbol ester stimulation (Patarroyo et al, 1984) (but not FMLP) also may increase adhesiveness (Detmers et al, 1988). That event is dependent on interaction of the receptor with the cytoskeleton, and like LFA-1, may be related to the phorbol ester-induced phosphorylation of the β_2 subunit (Buyon et al, 1990). Significantly, FMLP stimulates cellular aggregation with different kinetics

(rapid but transient) and does not induce phosphorylation. In addition, clustering of the ligand itself increases the avidity of macrophage and neutrophil binding (Hermanowski et al, 1988).

The adhesion of Mac-1 to C3bi mediates neutrophil and monocyte attachment to opsonised particles and may be involved in neutrophil adhesion to endothelium at sites of inflammation and complement deposition (Marks et al, 1989). However, the ligand for Mac-1 in neutrophil aggregation and adherence to protein-coated plastic is different and unknown, and the site involved on Mac-1 may be distinct from the C3bi-binding site (Dana et al, 1986, Corbi et al, 1988b). Other molecules can also attach to Mac-1, including Leishmania gp63 (Russell and Wright, 1988, Talamas-Rohana et al, 1990), lipopolysaccharide (Wright and Jong, 1986a), filamentous hemagglutinin of Bordetella pertussis (Relman et al, 1990), histoplasma capsulatum, fibrinogen, and inactivated coagulation factor X (Kuypers and Roos, 1989). One report suggests that Mac-1 interacts with the carboxyl terminus of the γ chain of fibrinogen, which is also one site where fibrinogen interacts with platelet GP IIb/IIIa (Wright et al, 1988). However, another study concluded that the Mac-1-binding site on fibrinogen contained neither this portion of the γ chain nor an RGD sequence (Altieri et al, 1990).

The binding of Mac-1 to its ligand can cause activation of the Mac-1-bearing cell (Kuypers and Roos, 1989), resulting in respiratory burst activity, phagocytosis, enzyme release, and production of leukotriene B_4. Immobilised antibody against CD11b induces monocytes to express cell-associated IL-1 (Couturier et al, 1990). These results suggest that Mac-1 can function as a signal-transducing molecule, in a similar fashion to LFA-1 and other integrins.

(4) p150,95

The third member of the β_2 integrin family was originally identified as a 150 kD band in immunoprecipitation studies with an anti-CD18 antibody on neutrophils (Sanchez-Madrid et al, 1983), but specific anti-CD11c antibodies were later isolated (Springer et al, 1986). The neutrophil expression of p150,95 is less than both Mac-1 and LFA-1 (Mac-1 is the predominant receptor), but they are approximately equal on monocytes. However, p150,95 increases upon differentiation into macrophages while Mac-1 decreases (Hogg et al, 1986, Myones et al, 1988).

The other major population expressing p150,95 is large granular lymphocytes. It is also a marker for hairy cell leukaemia and found on

activated leukaemic B lymphocytes and some T cell clones (Miller et al, 1986, Schwarting et al, 1985).

P150,95 has C3bi binding activity on neutrophils and monocytes, and is probably identical to complement receptor type 4 (CR4) (Frade et al, 1985, Myones et al, 1988). As p150,95 is the major C3 receptor on tissue macrophages, it may contribute most to their clearance of C3-opsonised particles and immune complexes. Like Mac-1, it is stored on intracellular vesicles and rapidly mobilised to the cell surface by chemotactic factors (Springer et al, 1986). It is unknown whether these stimulants also increase the avidity of p150,95 for its ligand.

Antibodies against p150,95 have relatively little effect on neutrophil function, possibly because of the much higher expression of Mac-1 (Anderson et al, 1986). In contrast, p150,95 makes a significant contribution to monocyte functions (Myones et al, 1988, Te Velde et al, 1987), and is involved in the conjugate formation and cytotoxic activity of those CTL clones that express it (Keizer et al, 1987).

(5) Leucocyte adhesion deficiency (LAD)

The rare disease called leucocyte adhesion deficiency (LAD) is due to a genetic defect in or absence of the β_2 subunits. As a result, all three receptors are ineffective or absent (reviewed Anderson et al, 1987). The degree of deficiency is designated as severe (<0.3% of normal expression) and moderate (5 to 10% of normal expression) (Springer et al, 1987), and patients with severe deficiency rarely survive beyond childhood. They are susceptible to recurrent life-threatening bacterial and fungal infections, and have progressive periodontitis, delayed separation of the umbilical stump, poor wound healing, lack of pus formation, and peripheral leucocytosis. Their neutrophils, monocytes, and lymphocytes have marked defects in both in vivo and in vitro adherence-dependent immune functions.

Crowley et al (1980) suggested that the disease was due to a defect in neutrophil adhesion, and demonstrated that the patient's neutrophils lacked a particular cell surface protein. The monoclonal antibody 60.3, which recognises an epitope on the β_2 chain, does not bind to patients' cells (Beatty et al, 1983, Beatty et al, 1984), and Mac-1 is the major protein deficient on the neutrophils (Dana et al, 1984). Incubation of leucocytes from normal volunteers with 60.3 produces functional abnormalities comparable to those of the patients' cells.

Five distinct β_2 subunit phenotypes have been defined in LAD patients (Kishimoto et al, 1987b). They include low or absent mRNA and the formation of mutant subunits that do not associate with the α subunits. Analysis of the sequence of the β subunits from two LAD patients with normal sized chains revealed different single amino acid substitutions in a region of the cDNA that is highly conserved between all integrin β subunits (Wardlaw et al, 1990). These two amino acids are normally constant in all integrin β subunits, and may lie in a site critical for correct association with the α subunit. Transfection of Epstein-Barr virus-transformed B lymphoblastoid cells from patients with LAD with cDNA of the normal β_2 subunit restores expression of the $\alpha\beta$ heterodimer and full adhesion function of these cells (Hibbs et al, 1990).

The features of this disease are mainly due to defects of neutrophil and monocyte function, and lymphocyte function is relatively spared (Arnaout et al, 1984, Anderson et al, 1987). Delayed-type hypersensitivity reactions in the skin are normal, as are alloantigen-induced cytolytic T lymphocyte and natural killer cell function. The small numbers of LFA-1 molecules expressed by the patients' lymphocytes may be sufficient for normal function, or other pathways of lymphocyte adhesion may compensate.

LAD can be cured by bone marrow transplantation, as the deficient molecules are confined to leucocytes and their haematopoietic precursor cells (Fischer et al, 1983). These LAD patients were unable to mount an allogeneic mixed lymphocyte response, and did not reject their grafts.

(6) Effects of antibodies against leucocyte adhesion molecules on pathological processes

The successful engraftment of non-HLA identical bone marrow into LAD patients suggested that LFA-1 may be important in graft rejection (Fischer et al, 1983). The same group found that a monoclonal antibody against CD11a dramatically improved the engraftment rate over historical controls of mismatched haploidentical related donor bone marrow in 7 patients with various immunodeficiencies (Wiskott-Aldrich syndrome, combined immunodeficiency, osteopetrosis) (Fischer et al, 1986). However, another group have noted poor engraftment in patients with leukaemia who were treated with an anti-CD18 antibody (Baume et al, 1989). These patients were older and arguably more immunocompetent. In murine models of allograft rejection, anti-CD11a antibody prolonged the survival of allogeneic P815

tumour grafts in A/J mice (Heagy et al, 1984), and decreased the rejection of T lymphocyte-depleted allogeneic bone marrow (van Dijken et al, 1990).

Murine graft-vs-host disease and delayed-type hypersensitivity responses are inhibited by antibodies against LFA-1 (and against CD4) (Shiohara et al, J.Immunol. 1988), suggesting that LFA-1 is involved in the epidermal invasion of T lymphocytes.

There is conflicting data on the role of LFA-1 in the metastatic potential of lymphomas. Clayberger et al (1987b) found that high grade lymphomas lack or express little LFA-1 by comparison with lower grade lymphomas, and that low expression of LFA-1 correlates with poor stimulation of both autologous and allogeneic T lymphocyte responses. They suggested that low LFA-1-bearing tumours might not initiate an effective immune response and so escape immune surveillance. Low levels of LFA-1 (as well as ICAM-1 and LFA-3) also correlate with decreased immune recognition of Burkitt's lymphoma cell lines (Gregory et al, 1988, Billaud et al, 1990). In contrast, antibody against LFA-1 inhibits the invasion of hepatocyte and fibroblast cultures by lymphoma cells, and mutant cells which lack LFA-1 have reduced invasiveness (Roos and Roossien, 1987, Roossien et al, 1989). This suggests that LFA-1 enhances the metastatic potential of these lymphoma cells.

The role of Mac-1 in vivo has been investigated in several models. The intravenous infusion of an anti-CD18 antibody, at the time of placing polyvinyl sponges containing Salmonella typhosa endotoxin subcutaneously into rabbits, inhibits in a dose-dependent manner the subsequent neutrophil emigration into the sponges (Price et al, 1987). This is probably mainly due to blockage of the Mac-1 on the neutrophils, as in vitro studies show that anti-CD11b antibodies are the most effective inhibitors of neutrophil aggregation, adhesion, and chemotaxis. The rabbits receiving the anti-CD18 antibody also developed a peripheral neutrophilia, possibly due to decreased margination. This neutrophilia has been observed in patients with LAD.

A number of studies have examined the effect of these antibodies on diseases that are largely mediated by neutrophils. Antibody against CD18 reduces the leucocyte infiltration, cerebrospinal fluid leucocytosis, and degree of tissue damage in a rabbit model of experimental meningitis (Tuoman et al, 1989). In another study, anti-CD18 decreases organ injury and prolongs survival in a rabbit model of haemorrhagic shock and resuscitation (Vedder et al, 1988b). These results suggest that much of the ischaemic injury is mediated by leucocytes, and occurs both during the ischaemic phase and following reperfusion. The same antibody attenuates reperfusion injury in a

rabbit ear model by inhibiting leucocyte adhesion (Vedder et al, 1990). An anti-CD11b monoclonal antibody decreases the pulmonary injury by activated neutrophils in isolated perfused rat lungs (Ismail et al, 1987). Infusion with another anti-CD11b monoclonal antibody reduces the size of myocardial infarction following induction of regional myocardial ischaemia in dogs (Simpson et al, 1988). Neutrophil accumulation within the myocardium is significantly reduced. The investigators attributed the myocardial sparing effect of the antibody to a reduction in the reperfusion injury.

In summary, the most promising strategy from these studies is the use of antibodies against CD11b or CD18 to reduce tissue damage that is mediated by neutrophils.

(7) CD2/LFA-3

The next cell-cell adhesion pathway to be discussed is that between CD2 on T lymphocytes and its ligand LFA-3, which is expressed by many cells. CD4 and CD8 also have some role in stabilising the adhesion of T lymphocytes to cells expressing MHC class II and class I molecules respectively, but will not be discussed further.

The original study that isolated a monoclonal antibody against human LFA-1 (Sanchez-Madrid et al, 1982) also found monoclonal antibodies against two other cell surface molecules (that they called LFA-2 and LFA-3) that inhibited cytotoxic T lymphocyte-mediated killing. Antibodies against all three molecules block cytolysis by preventing cell-target conjugate formation (Krensky et al, 1984), and so they appear to act as adhesion molecules. LFA-2 is now known as CD2, and is identical to the previously described sheep red blood cell receptor on human T lymphocytes (Krensky et al, 1983, Howard et al, 1981, Kamoun et al, 1981).

CD2 is expressed by T lymphocytes, large granular lymphocytes, and thymocytes (Krensky et al, 1983, Springer et al, 1987) and is one of the earliest differentiation antigens to appear in T-lymphocyte ontogeny. It is a glycoprotein with a molecular weight of 47 to 55 kD that varies in size according to different stages of activation and/or differentiation of the cells. This heterogeneity is thought to be due to glycosylation differences.

Anti-CD2 antibodies inhibit CTL-mediated killing by binding to the CTL rather that the target cell (Krensky et al, 1983). They also inhibit thymocyte binding to thymic epithelial cells (Volger et al, 1987), natural killer (NK) cell activity, rosetting of sheep red blood cells with human T lymphocytes, antigen-

independent conjugation of thymocytes, T lymphoblasts, and CTL to B lymphoblastoid cells and K562 cells, and proliferation of peripheral blood lymphocytes and T cell lines induced by phytohaemagglutinin and the MLC. The inhibition of proliferation is accompanied by a failure of the T lymphocytes to induce IL-2 mRNA, secrete IL-2, and express the IL-2 receptor (Springer et al, 1987, Krensky et al, 1983). The inhibitory effects of anti-CD2 antibodies are due to blocking of adhesion between the cells, and the LFA-1 and CD2 pathways each contribute about half to the conjugation between CTL and their target cells, so that combinations of saturating amounts of anti-LFA-1 and anti-CD2 antibodies completely block conjugation (Shaw et al, 1986, Shaw and Ginther Luce, 1987). Memory T lymphocytes express increased amounts of CD2, suggesting that the adhesion of CD2 to its ligand is important for the enhanced responsiveness of these cells (Sanders et al, 1988a, Sanders et al, 1988b).

Three functionally important epitopes have been defined on the human CD2 moleule. $T11_1$ and $T11_2$ epitopes are expressed on both resting and activated T lymphocytes, whereas $T11_3$ ($CD2_R$) is definitely present on activated T cells but less clearly present on resting cells (Meuer et al, 1984, Kabelitz, 1990). Sheep red blood cell rosetting is primarily mediated by the $T11_1$ epitope.

Combinations of monoclonal antibodies against $T11_2$ and $T11_3$ epitopes results in IL-2-dependent T cell proliferation (Meuer et al, 1986). This "alternative" pathway of T cell activation is distinct from the TCR-CD3 complex "classical" pathway. Stimulation of CD2 also augments the submitogenic activation of T lymphocytes via the TCR (Bierer et al, 1988a). Further evidence that the CD2 and TCR/CD3 activation pathways of T lymphocytes are interdependent is the demonstration that CD2 antigen can be specifically co-precipitated with the TCR/CD3 complex (Brown et al, 1989). The activation of the T lymphocytes proceeds via hydrolysis of membrane phosphoinositides which generates second messengers that mobilise intracellular calcium and stimulate protein phosphorylation (Pantaleo et al, 1987). One of the effects of triggering T lymphocytes through the CD2 molecule is the enhancement of LFA-1-mediated cell adhesion. This results from an increase in the LFA-1 affinity for its ligand rather than an increase in the number of LFA-1 molecules (van Kooyk et al, 1989). CD2 has a large cytoplasmic tail (126 amino acids) which may be important in the delivery of these CD2-dependent activation signals into the cell (He et al, 1988).

The physiological relevance of these observations may be that the binding of CD2 to its ligand on the target cell enhances both the activation of the T lymphocyte as well as the adhesion between the cells.

The CD2 ligand is LFA-3, the other molecule identified by Sandez-Madrid et al (1982). Like antibodies against CD2, anti-LFA-3 antibodies block conjugate formation (Krensky et al, 1984), but antibodies against CD2 and LFA-3 do not have additive effects, which suggested that they are involved in the same adhesion pathway (Shaw et al, 1986). Antibodies against LFA-3 inhibit CD2-mediated functions by binding to the target cell rather than the effector cell (Krensky et al, 1983). A number of studies have now shown that LFA-3 binds specifically to CD2 (Selvaraj et al, 1987a, Dustin et al, 1987a, Takai et al, 1987), and that it is the human equivalent of the sheep red blood cell receptor for CD2 (Makgoba et al, 1987, Plunkett et al, 1987, Hunig et al, 1987). In contrast to adhesion involving members of the integrin family, CD2/LFA-3 adhesion is neither dependent on divalent cations nor temperature sensitive (Shaw et al, 1986).

LFA-3 is expressed by most cell types, including leucocytes, platelets, erythrocytes, endothelial cells, smooth muscle cells, and fibroblasts (Krensky et al, 1983). It has a molecular weight of 55 to 70 kD depending on the cell type. The cDNA clone defines a mature protein of 222 amino acids, with an extracellular domain containing 6 N-linked glycosylation sites, a short hydrophilic transmembrane region, and a short cytoplasmic domain (Wallner et al, 1987). Both LFA-3 and CD2 are members of the immunoglobulin supergene family (Williams and Barclay, 1988). An alternative form of LFA-3 is anchored to the cell membrane via a phosphatidyl inositol glycan moiety (Dustin et al, 1987b). This may mediate the adhesive function of LFA-3 as it probably has faster lateral mobility in the cell membrane, which would aid diffusion of the LFA-3 molecules to sites of adhesion with cells expressing CD2. Consistent with this finding, LFA-3 is deficient in erythrocytes from patients with paroxysmal nocturnal haemoglobinuria, a disorder affecting phophatidyl inositol-linked proteins (Selvaraj et al, 1987b).

The binding of LFA-3 to CD2 provides a partial activation signal for the T lymphocyte (Hunig et al, 1987, Bierer et al, 1988b, Webb et al, 1990) and facilitates T lymphocyte antigen recognition function (Moingeon et al, 1989). This requires cell surface expression of a functional TCR/CD3 complex (Bockenstedt et al, 1988). The effect of LFA-3 on the CD2-expressing cell may be both mediated by signals transduced by CD2, and by factors generated by the LFA-3-bearing cell as a result of the CD2 binding. For example, anti-LFA-3

antibodies induce monocytes to release TNF (Webb et al, 1990) and thymic epithelial cells and monocytes to release IL-1 (Le et al, 1990), and endothelial cells augment T lymphocyte IL-2 production by a contact-dependent mechanism that involves the CD2/LFA-3 interaction (Hughes et al, 1990).

It is clear that the adhesion of both LFA-1 and CD2 to their ligands on cells such as antigen-presenting cells does more than just "glue" the cells together. Interaction of LFA-1 with its ligand activates the T lymphocyte, which in turn "cements" the bond by enhancing the avidity of LFA-1 for its ligand. The binding of CD2 to LFA-3 stimulates the T lymphocyte directly via CD2, and indirectly by the release of factors by the APC as a result of LFA-3 stimulation. It is possible that the CD2/LFA-3 interaction also stimulates the other "co-stimulatory signals" (see section 2) that are necessary for complete antigen-presenting function. The increased expression by memory T cells of LFA-1, CD2 and also LFA-3 (Sanders et al, 1988a) is therefore not surprising, considering that these cells have enhanced abilities to respond to antigen. They also exhibit increased adhesiveness in vitro (Pitzalis et al, 1988), which may underly their preferential accumulation at sites of inflammation in autoimmune disease (Sanders at al, 1988b).

The multiplicity of signals provided by adhesion molecules, plus the antigen-specific signal to the TCR from the MHC-antigen complex and the interaction between CD4 or CD8 molecules on the T cell and the MHC molecules, represents an extremely complex set of interactions when T lymphocytes encounter their targets. There is evidence from monoclonal antibody blocking experiments that all of these pathways have some role in the regulation of this event.

Endothelial Adhesion Receptors for Leucocytes

The rest of this literature review will cover the adhesion molecules expressed by endothelial cells that are relevant to their adhesiveness for leucocytes.

(1) Vascular addressins

HEV must express tissue-specific receptors for the lymphocyte homing receptors to enable the lymphocytes to distinguish between HEV in different sites. Monoclonal antibodies have been developed which recognise these HEV-specific antigens, and they are called "vascular addressins", because

they guide the lymphocytes to a particular site ("address") (Streeter et al, 1988a).

The monoclonal antibodies MECA-89 and MECA-367 selectively bind to HEV in murine mucosal lymphoid tissue, such as Peyer's patches (Streeter et al, 1988a). MECA-367 blocks the adhesion of lymphocytes to Peyer's patch HEV in the in vitro assay used by this group, and in vivo it inhibits the entry of radiolabelled tranfused lymphocytes into mucosal lymphoid tissue. It also partially blocks neutrophil adhesion to Peyer's patch HEV (Jutila et al, 1989a). MECA-89 binds to a distinct epitope on the same molecule but does not inhibit adhesion. This "mucosal addressin" is a single-chain protein with a molecular weight of 58 to 66 kD reduced and 54 to 62 kD non-reduced. Isoelectric focussing shows that it has significant charge heterogeneity. Purified antigen inserted into supported phospholipid planar membranes binds lymphocytes specifically, and only those lymphocytes or lymphoma cell lines capable of binding to mucosal HEV adhere well (Nakache et al, 1989). The lymphocyte receptor for the mucosal addressin is probably CD44 (Hermes), as the monoclonal antibodies Hermes-3 and MECA-367 specifically inhibit the binding of purified CD44 antigen to purified mucosal addressin (Berg et al, 1989).

Another monoclonal antibody, named MECA-79, binds strongly to HEV in murine peripheral lymph nodes, and weakly to HEV in other sites (Streeter et al, 1988b). It blocks the adhesion of normal lymphocytes and a peripheral lymph node-specific lymphoma to peripheral lymph node HEV in vitro, and inhibits normal lymphocyte homing to peripheral lymph nodes in vivo. It partially inhibits neutrophil adhesion to peripheral lymph node HEV (Jutila et al, 1989a). In humans, MECA-79 stains venules in cutaneous sites of chronic inflammation (Jutila et al, 1989a). It recognises a 92 kD single chain glycoprotein, which is sensitive to N-glycanase digestion (Berg et al, 1989). It is possible that this antigen is the HEV receptor for the MEL-14 homing receptor on lymphocytes, but this has not been proven.

The MECA-325 antibody defines an endothelial cell differentiation antigen that is selectively expressed on high endothelium in the mouse (Duijvestijn et al, 1987). The antigen appears on HEV-like vessels that develop in induced subcutaneous granulomas and in sponge matrix allografts (Bishop et al, 1989), in parallel with the appearance of a mononuclear infiltrate which includes numerous lymphocytes. It is selectively induced on cultured murine lung and bone marrow endothelium by γ-IF (Duijvestijn et al, 1986). This is further evidence that the development of HEV-like vessels in vivo is

dependent on local factors such as cytokine release by infiltrating mononuclear cells. No information is available on the structure of the MECA-325 antigen, and the antibody has no effect on lymphocyte adhesion to HEV. This could be because the antigen is only a marker of the HEV phenotype rather than directly involved in lymphocyte adhesion, or that the antibody binds to a non-functional epitope.

The MECA-32 antibody recognises an antigen that is not normally present on murine cardiac endothelium, but is strongly expressed by the capillaries and venules of rejecting cardiac allografts (Leppink et al, 1989). These vessels frequently have mononuclear cells adherent to the intima and perivascular cuffs of mononuclear cells. Induction of myocarditis in non-transplanted hearts with cocksackie virus is also associated with MECA-32 expression in the vessels that are surrounded by a mononuclear infiltrate. Hence, allorecognition and virus-induced inflammation, both of which are associated with a mononuclear infiltrate, induce the MECA-32 antigen. However, there is no direct evidence that it is involved in lymphocyte adhesion and extravasation, and its structure is unknown.

The only monoclonal antibody that recognises a human HEV-specific antigen is HECA-452 (Duijvestijn et al, 1988). It stains HEV in all lymphoid organs and at sites of chronic inflammation, as well as cross-reacting with a subset of monocytic cells. The antigen is inducible on cultured human endothelial cells by γ-IF. HECA-452 has no effect on lymphocyte adhesion to HEV, so it is unknown whether it defines an adhesion-related molecule or is simply a marker for HEV. The antigen has a molecular weight of 190 to 240 kD, but no other structural information has been published (Duijvestijn and Hamann, 1989).

While the vascular addressins are tissue and/or HEV specific, a number of other endothelial adhesion molecules have been described that are more widely distributed or appear following other types of activation.

(2) Intercellular adhesion molecule-1 (ICAM-1)

Epstein-Barr virus (EBV)-transformed B lymphocytes from LFA-1-deficient patients do not self-aggregate but can form conjugates with cells expressing LFA-1, which implies that LFA-1 mediates adhesion by binding to a ligand distinct from itself. Neither CD2 nor LFA-3 are ligands for LFA-1 (Shaw et al, 1986). A monoclonal antibody (RR1/1) which inhibits phorbol ester-stimulated

aggregation of LFA-1-positive cells was produced by immunisation with LFA-1-deficient EBV-transformed lymphoblastoid cells (Rothlein et al, 1986b). The molecule recognised by the antibody was distinct from other adhesion molecules and was named intercellular adhesion molecule-1 (ICAM-1). It is identical to the antigen expressed by activated B lymphocytes identified by the monoclonal antibody LB-2 (Clark et al, 1986), and has now been designated CD54.

The murine equivalent of human ICAM-1 is recognised by the rat monoclonal antibody YN1/1.7, which inhibits the mixed lymphocyte response (Takei, 1985). The gene for murine ICAM-1 has also been isolated, but the sequence has limited homology (50%) to human ICAM-1 (Siu et al, 1989, Horley et al, 1989). However, murine cell lines still bind to purified human LFA-1 through their ICAM-1, although murine LFA-1 does not bind to human ICAM-1 (Johnston et al, 1990). The specificity of the LFA-1 between these two species maps to its α subunit.

The molecular weight of human ICAM-1 varies between cell types. It is 97 kD on fibroblasts, 114 kD on U937 myelomonocytic cells, and 90 kD on JY B lymphoblastoid cells (Dustin et al, 1986). On cultured HUVEC it has a molecular weight of 100 kD reduced and 96 kD non-reduced, which suggests that it has intrachain disulphide bonds (Staunton et al, 1988). The heterogeneity between cells is due to differences in glycosylation, and treatment with tunicamycin (which removes N-glycosylated residues) leaves a 55 kD form (Dustin et al, 1986). This size is in close agreement with the predicted size of the mature polypeptide chain from sequencing data (Staunton et al, 1988). ICAM-1 is a typical integral membrane protein with a 453 amino acid extracellular sequence, a 24 residue hydrophilic transmembrane sequence, and a 28 residue cytoplasmic domain. It is initially synthesised as a 73 kD precursor, which is also in good agreement with the predicted size if all 8 potential N-glycosylation sites are used. The mature glycoprotein is then formed by conversion of the high mannose N-linked carbohydrate to complex carbohydrate, and the final form depends on cell type.

The amino acid sequence of ICAM-1 contains five extracellular immunoglobulin-like domains, which identifies it as a member of the immunoglobulin superfamily (reviewed Williams and Barclay, 1988). It has no significant homology with members of the integrin family. ICAM-1 has the most homology with two adhesion proteins of the adult nervous system, neural cell adhesion molecule (NCAM) (20%) and myelin associated glycoprotein (MAG)

(24%), both of which also have five immunoglobulin-like domains (Simmons et al, 1988, Staunton et al, 1988, Dustin et al, 1988a).

The immunoglobulin-like domain is a basic structural unit of about 100 amino acids based on the domains originally described in the V and C regions of immunoglobulin heavy and light chains. It is formed by a characteristic fold that results in a sandwich of two β-pleated sheets stabilised by a conserved disulphide bond. The two sheets consist of anti-parallel β-strands containing 5 to 10 amino acids. Structure based on this unit is the characteristic of members of the immunoglobulin superfamily. These molecules generally have a role in cell surface recognition and are often involved in cell-cell adhesion. It is commonly accepted that they derived by gene duplication and divergance from one primordial domain. The family is large and new members are regularly being found. They include the following molecules: TCR complex, CD3 complex, MHC class I and class II, β_2-microglobulin, CD1, CD2, LFA-3, CD4, CD8, Thy-1, poly Ig receptor, carcinoembryonic antigen, platelet-derived growth factor (PDGF) receptor, and colony stimulating factor-1, plus ICAM-1, NCAM, and MAG.

The anti-ICAM-1 antibody RR1/1 inhibited phorbol ester-stimulated LFA-1-dependent adhesion of B lymphocyte and myeloid cell lines and T lymphocyte blasts, although the aggregation of one T lymphocyte line (SKW-3) was inhibited by anti-LFA-1 but not by anti-ICAM-1 (Rothlein et al, 1986b). These observations suggested that ICAM-1 is the ligand for LFA-1 in most, but not all, LFA-1-dependent interactions. This was proven by the specific adhesion of LFA-1-positive B, T, and myeloid cells to purified ICAM-1 incorporated into artificial supported lipid membranes (Marlin and Springer, 1987) and coated onto plastic (Makgoba et al, 1988a), and to ICAM-1-expressing L-cell transfectants (Wawryk et al, 1989). These interactions require metabolic energy production, an intact cytoskeleton, the presence of Mg^{++}, and are temperature dependent, which is typical of the adhesion of integrins to their ligands. The adhesion is not inhibited by RGD-containing peptides (Marlin and Springer, 1987), and the primary sequence of ICAM-1 does not contain the RGD tripeptide. The recognition of LFA-1 and ICAM-1 as a ligand pair was the first example of interaction between members of the integrin and immunoglobulin families.

Antibodies against ICAM-1 inhibit LFA-1-dependent adhesion by binding to ICAM-1 on the target cell (Makgoba et al, 1988b). ICAM-1-dependent interactions include aggregation of leucocytes and leucocyte cell lines, the mixed lymphocyte reaction (MLR), autologous mixed lymphocyte reaction, T

lymphocyte-mediated B lymphocyte activation, lymphocyte activation by mitogens, T lymphocyte proliferation in response to soluble antigen, natural killer (NK) cell function, and lymphokine-activated killer (LAK) cell-mediated cytotoxicity, all of which require cell-cell adhesion (Rothlein et al, 1986b, Timonen et al, 1988, Dougherty et al, 1988, Wawryk et al, 1989, Boyd et al, 1988, Maio et al, 1989, Bagnasco et al, 1990). In contrast, the non-adhesion-dependent activation of lymphocyte blasts by purified cytokines is not affected by antibodies against ICAM-1 (Boyd et al, 1988). There is evidence that different sites on ICAM-1 mediate some of its functions (Maio et al, 1989).

The expression of ICAM-1 is essential for the complete functioning of antigen-presenting cells (Altmann et al, 1989), and reconstitution by transfection of ICAM-1 into mutant antigen presenting cells that constitutively lack ICAM-1 fully restores their ability to present antigen (Dang et al, 1990). The interaction of ICAM-1 with LFA-1 transmits regulatory signals to the T lymphocyte (van Noesel et al, 1988, van Seventer et al, 1990). These signals are synergistic with the activation induced by the binding of the MHC/antigen complex to the TCR/CD3 complex. However, while LFA-1 becomes more avid after cell activation, ICAM-1 is constitutively avid for LFA-1 and this does not undergo further regulation (Dustin and Springer, 1989). There has been no published evidence that engagement of ICAM-1 by LFA-1 directly results in any changes in the ICAM-1-bearing cell. The level of expression of ICAM-1 by cells is under the control of cytokines secreted by activated mononuclear cells, providing extra potential ligands for the more avid LFA-1.

ICAM-1 expression is increased by inflammatory stimuli and is a marker of cellular activation. Peripheral blood leucocytes, fibroblasts, and cultured HUVEC express it in low amounts, whereas it is found in greater density on mitogen-activated T lymphocytes, EBV-transformed B lymphocytes (especially those that spontaneously aggregate), and on some T lymphocytic and myelomonocytic cell lines (Rothlein et al, 1986b, Dustin et al, 1986, Wawryk et al, 1989). The constitutive expression of ICAM-1 on cultured HUVEC is inhibited by IL-4 (Thornhill et al, 1990). ICAM-1 is absent from normal foetal and adult brain, but is significantly expressed on cultured human glioblastoma cells and on intratumoural vascular endothelial cells (Kuppner et al, 1990). It is significantly upregulated in the following situations: activation of peripheral blood leucocytes; stimulation of fibroblasts and human carcinoma cell lines with IL-1, TNF, or γ-IF; stimulation of cultured HUVEC with IL-1, TNF, LT, γ-IF, or LPS (also inhibited by IL-4 [Thornhill et al, 1990]); stimulation of cultured glioblastoma cells with IL-1, TNF, and γ-IF; and phorbol ester-induced

differentiation of myelomonocytic cell lines (Rothlein et al, 1988, Dustin et al, 1986, Warwyk et al, 1989, Pober et al, 1986c, Kuppner et al, 1990, Pober et al, 1987). The increased expression of ICAM-1 depends on mRNA and protein synthesis, and persists while the stimulus continues, but is reversible upon its removal.

ICAM-1 is normally expressed in tissue sections on vascular endothelium, tissue macrophages, germinal centre dendritic cells, and thymic and mucosal epithelial cells (Dustin et al, 1986). It is increased at sites of inflammation, including rheumatoid synovium and inflammatory skin disorders (Hale et al, 1989a, Wantzin et al, 1988, Griffiths et al, 1989, Lisby et al, 1989). Studies to be described in this thesis show the upregulation of ICAM-1 in rejecting renal allografts. This increased expression suggests that ICAM-1 regulates the adherence of leucocytes during inflammation and immune responses, and it is probable that it is caused by cytokines released by the infiltrating activated leucocytes.

The role of ICAM-1 in inflammation has been studied in detail in the skin. In the normal human skin, it is confined to the dermal vascular endothelium in low amounts. In a variety of skin disorders the endothelial expression increases and ICAM-1 also appears on the epidermal keratinocytes, in association with focal infiltrates of inflammatory cells (Wantzin et al, 1988, Griffiths et al, 1989, Lisby et al, 1989). A parallel increase in the expression of HLA class II molecules also occurs.

Keratinocytes in culture normally express little or no ICAM-1, but it is readily induced by γ-IF and to a lesser extent by TNF (Griffiths et al, 1989, Dustin et al, 1988b). T lymphoblasts will only adhere to keratinocytes after they have been stimulated, and this is inhibited by antibodies against LFA-1 or ICAM-1.

Intracutaneous injection of γ-IF into baboon skin is followed by increased endothelial MHC class II expression and a mild to moderate accumulation of mononuclear cells (Munro et al, 1989). TNF alone induces ELAM-1 (see later) and ICAM-1 plus a mononuclear cell infiltrate. When both are injected, the keratinocytes are also induced to express ICAM-1 and the histological changes resemble a delayed-type hypersensitivity reaction. Similar results are obtained with γ-IF and IL-1 injected into rat skin (Issekutz et al, 1988).

These studies show that cytokines can generate and amplify a mononuclear infiltrate in the skin. However, the stimulus that initially activates the T lymphocytes and so commences the response is unknown in most inflammatory skin diseases. It is likely that APCs bearing the presumed

causative antigen activate the appropriate helper T lymphocytes, which in turn produce cytokines and stimulate their production by other cells. The initiating APC may be the endothelial cells as only they normally express MHC class II antigens and ICAM-1, both of which seem to be necessary for efficient antigen presentation. Interstitial dendritic cells are traditionally regarded as the chief APCs in the skin, but they do not normally express ICAM-1 (Griffiths et al, 1989). Once the inflammatory cascade has been initiated, endothelial cells, dendritic cells, and keratinocytes all express MHC class II and ICAM-1, and so all may actively participate in the immune response. γ-IF is probably the most important cytokine in the initiation and maintenance of this process (Nickoloff 1988).

Another skin disease involving ICAM-1 is malignant melanoma. The melanoma progression-associated antigen P3.58 is identical to ICAM-1 (Johnson et al, 1988) and is found on advanced melanomas but not on benign melanocytes or early melanomas. It correlates with increased risk of metastasis as well as the local presence of activated leucocytes that are producing γ-IF (Johnson et al, 1989), and probably represents a marker of the patient's immune response to the malignant cells.

Although antibodies against ICAM-1 inhibit multiple immune functions, there have been few published studies of their therapeutic use in inflammatory diseases. They inhibit neutrophil migration into phorbol ester-induced inflamed rabbit lungs (Barton et al, 1989), and attenuate airway eosinophilia and hyperresponsiveness in a primate model of asthma (Wegner et al, 1990). In this study, ICAM-1 expression was upregulated on the inflamed airway epithelium in vitro and in vivo. Antibody against human ICAM-1 cross-reacts with monkey ICAM-1, and delayed the onset of acute cellular rejection and reversed established acute rejection in primate renal allografts (Cosimi et al, 1990). Notably there was an almost complete lack of vascular rejection. The investigators hypothesised that the beneficial effects of the antibody were due to it blocking the adhesion of host effector cells to the endothelium in the allograft, although it may also interfere with antigen presentation or other T lymphocyte interactions.

ICAM-1 is also a cell surface ligand for two important pathogens, the Rhinoviruses and Plasmodium falciparum.

Rhinoviruses are a major cause of the common cold. About 90% of Rhinovirus serotypes bind to ICAM-1 on the target cell, and also bind to ICAM-1 transfected into COS cells (Staunton et al, 1989a, Greve et al, 1989). This adhesion is specifically inhibited only by anti-ICAM-1 antibodies which also

block LFA-1-dependent adhesion, which implies that the site for rhinovirus adhesion is in close proximity to the binding site for LFA-1. Site specific mutagenesis and truncation of the ICAM-1 molecule have shown that the binding sites are distinct but overlapping, and that rhinovirus binding does not require divalent cations (Marlin et al, 1990, Staunton et al, 1990).

The extracellular portion of ICAM-1 has been constructed and purified. It specifically inhibits the adhesion of rhinovirus to the target cell, and is being actively investigated as a potential therapy for the common cold (Marlin et al, 1990, Bangham and McMichael, 1990).

ICAM-1 is homologous to the cell surface receptors for human immunodeficiency virus (which is CD4) and poliovirus (Mendelsohn et al, 1989, White and Littman, 1989). All three receptors have the conserved amino acids and domain structure characteristic of the immunoglobulin superfamily.

Plasmodium falciparum-infected red blood cells adhere to the endothelium of small blood vessels in a process called sequestration, and so avoid removal by the spleen. This event may also contribute to the complications and mortality of severe malaria, particularly by obstructing the cerebral capillaries.

Three receptors on the endothelium for these infected erythrocytes have been identified. They are thrombospondin (Roberts et al, 1985), CD36 (also known as platelet glycoprotein IV) (Barnwell et al, 1985, Ockenhouse et al, 1989, Oquendo et al, 1989), and ICAM-1 (Berendt et al, 1989). CD36 is also the cell membrane receptor for thrombospondin (Asch et al, 1987). The relative roles of each receptor in the adhesion of infected erythrocytes to the endothelium in clinical malaria are uncertain, but ICAM-1 may be particularly important where cytokines are produced (eg where there are mononuclear cell infiltrates), as this will increase its expression on the endothelium (Miller, 1989).

(3) Selectins

The selectins are another family of cell surface molecules that are involved in intercellular adhesion. Two members are the lymphocyte homing receptors MEL-14 and LAM-1, which have been discussed in the section on HEV and lymphocyte recirculation. The other two members are expressed by endothelial cells and will be discussed in this section. They both resemble ICAM-1 in that they are involved in adhesion and also signify endothelial activation.

The selectins are distinguished by their unusual and characteristic chimeric structure composed of an amino-terminal lectin-like domain, followed by an epidermal growth factor-like domain, and then a series of tandem repetitive motifs which are related to the complement regulatory proteins. The name "selectins" refers to their lectin domain and their role in selective cell trafficking (Tedder et al, 1990). They have no homology to the integrin or immunoglobulin families, and do not possess RGD recognition sequences. The genes encoding the selectins are clustered together on mouse and human chromosome 1, suggesting that they arose by multiple gene duplication events before the evolutionary divergance of mouse and humans (Watson et al, 1990).

Endothelial leucocyte adhesion molecule-1 (ELAM-1)

Neutrophils, monocytes, and myeloid cell lines exhibit increased adherence to IL-1-stimulated cultured HUVEC (Bevilacqua et al, 1985a). This is mediated by an inducible endothelial adhesion molecule that was named ELAM-1 following its identification by the monoclonal antibody H4/18 (Pober et al, 1986b). ELAM-1 is confined to endothelial cells, but only appears on cultured HUVEC after stimulation with IL-1, TNF, LT, LPS, and phorbol esters (transiently) (Pober et al, 1986b, Pober et al, 1987, Bevilacqua et al, 1987). It is not upregulated by γ-IF (unlike ICAM-1) (Pober et al, 1986b), but γ-IF does enhance and prolong the expression of ELAM-1 once it has been induced by these other factors (Leeuwenberg et al, 1990). The induction of ELAM-1 is inhibited by IL-4 (Thornhill et al, 1990).

Peak expression of ELAM-1 occurs after 4 to 6 hours of stimulation, and is dependent on mRNA and protein synthesis. It declines to near basal levels after 24 hours even in the presence of continued stimulation, which is in contrast to ICAM-1. The cells can be re-stimulated to express ELAM-1 if rested for 20 hours, and HUVEC that are refractory to IL-1 stimulation can be induced to express it by TNF, and vice versa, suggesting that IL-1 and TNF act independently on the HUVEC.

Antibodies against ELAM-1 immunoprecipitate two biosynthetically labelled polypeptides (100 kD and 120 kD) from stimulated HUVEC. Treatment of the immunoprecipitates with N-glycosidase F to remove N-linked carbohydrates yields a single 78 kD band (Bevilacqua et al, 1989), so it appears that the two bands are the result of glycosylation differences. The predicted sequence of ELAM-1 from the cDNA contains a 32 amino acid

cytoplasmic domain, a 22 amino acid transmembrane portion, and a 535 residue extracellular domain with 11 potential N-linked glycosylation sites (Bevilacqua et al, 1989).

The kinetics of expression of ELAM-1 correlates with the cytokine-induced increased adhesiveness for leucocytes, and antibodies against ELAM-1 partially inhibit adhesion of neutrophils and the myelomonocytic cell line HL60 to stimulated HUVEC (Bevilacqua et al, 1987). They have a lesser effect on the adhesion of monocytes and lymphocytes (Bevilacqua et al, 1989). The early but transient expression of ELAM-1 is consistent with the migration of neutrophils into acute inflammatory lesions, whereas the more prolonged upregulation of ICAM-1 after stimulation suggests a role for it in lymphocyte and monocyte extravasation into chronic inflammatory lesions. COS cells expressing ELAM-1 bind HL60 by a Ca^{++}-dependent but temperature insensitive process that is not inhibited by simple carbohydrate structures (Hession et al, 1990). Like MEL-14, the ligand for ELAM-1 is unknown. It is most likely to be a complex carbohydrate if the adhesion depends on the amino-terminal lectin-like structure. Anti-β_2 integrin antibodies have no effect on the binding of neutrophils or HL60 to COS cells that express ELAM-1, so the leucocyte structure that binds to ELAM-1 is not a member of the β_2 integrin family.

The transience of ELAM-1 expression makes it a useful marker of acute inflammatory reactions. It is present in acute dermatological conditions but disappears after several days in delayed-type hypersensitivity reactions, and is not found in chronic inflammatory conditions (Cotran et al, 1986, Pober, 1988). In contrast, the elevated expression of HLA class II antigens and ICAM-1 tends to persist in chronic inflammation. Intracutaneous injection of TNF into baboon skin induces an early (2 hours) expression of ELAM-1 accompanied by a neutrophil infiltrate, whereas ICAM-1 increases later (9 hours) associated with a progressive mononuclear cell infiltrate (Munro et al, 1989). Cultured newborn foreskins stimulated with cytokines transiently express ELAM-1, predominantly on the postcapillary venular endothelial cells of the superficial vascular plexus, which is the area of the skin most involved in leucocyte and lymphocyte trafficking (Messadi et al, 1987). Degranulation of mast cells in skin organ cultures by morphine sulphate induces ELAM-1 on the adjacent vessels within 2 hours (Klein et al, 1989). This is blocked by preincubation with chromolyn sodium (an inhibitor of mast cell secretion) or antiserum to TNF. ELAM-1 appears on cultured glomerular endothelium after stimulation with IL-1 or LPS, but disappears by 24 hours (Hancock and Cotran, 1987).

This transient expression of ELAM-1 may explain why it has not been found in many disorders. It is not present on the endothelium of normal tissues (Cotran et al, 1986), and renal biosies taken from patients with various glomerular diseases show minimal focal expression (Hancock and Cotran, 1987). In a study of a number of pathological tissues, ELAM-1 was found on the endothelium of a lymph node from a patient with angioimmunoblastic lymphadenopathy with developing T cell lymphoma (Cotran et al, 1986). The staining was most prominent on the hyperplastic venules characteristic of this disease. It was also found in Hodgkin's disease (again on hyperplastic vessels), acute granulomatous lymphadenitis, lymphocyte-rich thyroiditis, hyperplastic tonsillitis, acute appendicitis, and active inflammatory dermatitis. It was absent in B cell lymphoma, dermatopathic lymphadenitis, chronic sarcoid granuloma, renal allograft rejection, foreign body granuloma, chronic pneumonitis, viral hepatitis, and around burn wounds.

A number of patients receiving IL-2 immunotherapy for metastatic cancer develop a vascular leak syndrome. The endothelium in biopsies from their skin expresses large amounts of ELAM-1, ICAM-1, and HLA class II (Cotran et al, 1987). The activation and "leakiness" of the endothelium probably develops as a result of cytokines produced by cells stimulated by the IL-2, as IL-2 itself does not upregulate these antigens on cultured endothelial cells in vitro.

Granule membrane protein-140 (GMP-140)

Neutrophils rapidly (within minutes) adhere to the endothelium after injury. This is not accounted for by ELAM-1 or ICAM-1 as their expression on stimulated HUVEC requires new protein synthesis and takes several hours. However, neutrophils rapidly adhere to cultured HUVEC stimulated by thrombin (Zimmerman et al, 1985). The adhesion is independent of the CD18 molecules on the neutrophils (Zimmerman and McIntyre, 1988), and is mediated by GMP-140 on the endothelial cells (Geng et al, 1990). GMP-140 (also known as platelet activation-dependent granule to external membrane protein [PADGEM]) is localised in the Weibel-Palade bodies of the endothelial cells, along with von Willebrand factor (McEver et al, 1989, Bonfanti et al, 1989), but is rapidly translocated to the cell surface following stimulation with histamine, thrombin, or phorbol esters. It was originally described as a protein found on the membranes of the α-granules of human platelets that was rapidly expressed on the cell surface following activation (McEver and Martin, 1984).

GMP-140 is also found in megakaryocytes and the HEL cell line (Johnston et al, 1989a).

Tunicamycin-treated HEL cells synthesise several 80 to 92 kD precursors of GMP-140, suggesting that there is heterogeneity of the protein sequence (Johnston et al, 1989a). Isolated cDNA either encodes a complete membrane spanning protein or a soluble form with a deleted transmembrane segment (Johnston et al, 1989b). The predicted primary sequence has the characteristic set of domains found in other members of the selectin family, and 12 potential N-linked glycosylation sites.

Platelet GMP-140 is responsible for the adhesion of activated platelets to neutrophils and monocytes (Larsen et al, 1989, Hamburger et al, 1990). This may be to facilitate the clearance of these thrombogenic cells.

Endothelial cell GMP-140 probably exists for rapid neutrophil adherence at sites of injury, followed by more sustained ELAM-1-induced adherence if the appropriate signals are present. The predicted soluble form of GMP-140 could have a regulatory function by binding to neutrophils and blocking the ligand for the membrane-bound GMP-140. In one study, soluble GMP-140 inhibited the CD18-dependent adherence of TNF-activated neutrophils to cultured HUVEC (Gamble et al, 1990).

Adhesion mediated by GMP-140 is divalent cation-dependent. The ligand is unknown, but it is likely to be a carbohydrate structure that binds to the lectin-like domain.

4.2: Studies of leucocyte adhesion to cultured HUVEC

The literature review at the commencement of this section dealt with many aspects of the adhesion molecules that are important for leucocyte adhesion to the endothelium. The next part focusses on the work involved in setting up an assay for quantifying leucocyte adhesion to cultured HUVEC. The initial work standardising the assay was performed with human peripheral blood neutrophils and related cell lines, and this is presented with a literature review of relevant studies using these cells. The bulk of the work involved human peripheral blood T lymphocytes and related cell lines, as these are the cells more directly relevant to allograft rejection, and this is also presented accompanied by a review of studies performed by other groups.

Several different assays have been developed to quantify the adhesion of leucocytes to monolayers of cultured endothelial cells, but they all basically rely on incubating the cells together for a defined period, and then removing the non-adherent leucocytes by a variety of washing procedures. The assay conditions can be modified to examine different aspects of the adhesion process. For example, the leucocytes and/or the endothelial cells may be stimulated, the composition of the medium altered, or antibodies introduced to attempt to inhibit the adhesion. More sophisticated techniques are necessary to examine adhesion under flow conditions, but this is arguably a more physiological situation.

The static adhesion assay used in this thesis is based on the quantitative assay for intercellular adhesion devised by Walther et al (1973). Various modifications of this method have been used by other investigators, including manual counting of the adherent cells, labelling the cells with ^{51}Chromium (or other radioactive label), and removal of the non-adherent cells by inverted centrifugation (Hoover et al, 1978, Zweiman et al, 1982, Charo et al, 1985). The last modification was used in this project because preliminary studies for this thesis showed that it reduces variability in the removal of the non-adherent cells.

Two interesting variations of the basic assay have also been developed. The leucocytes can be labelled with a monoclonal antibody/peroxidase conjugate and the adherent cells measured by a simple ELISA (Keizer et al, 1986). Alternatively, the leucocytes and cultured HUVEC are labelled with the vital stain Rose Bengal in the well after the adhesion step. The adherent cells are quantified by an ELISA readout after release of the dye

by cell lysis, and subtracting the readout from that obtained in wells containing HUVEC alone (Gamble and Vadas, 1988a).

4.2 (1) Adhesion of neutrophils and HL60 cells to cultured HUVEC

Neutrophils were purified from the peripheral blood of normal donors for use in the adhesion assay. Approximately 5% of the unstimulated neutrophils added to a well remain adherent to unstimulated HUVEC. Under the same assay conditions, less than 1% of unstimulated HL60 cells adhere to unstimulated HUVEC (see graphs page 221).

A rapid (within minutes) increase in adhesion of neutrophils and HL60 occurs if they are stimulated with PMA. A plateau of 50 to 80% of the added neutrophils adhered to the HUVEC at a concentration of 30 ng/ml of PMA, but the maximum number of HL60 cells remaining adherent was 3 to 4% (graphs page 221).

Unstimulated neutrophils also adhere in greater numbers to cultured HUVEC which have been stimulated with LPS. In contrast to the increased adhesion seen with PMA stimulation of the neutrophils, this increase is slower in onset (detectable at about 1 hour), and peaks after about 3 to 4 hours of stimulation. A maximum of 50 to 60% of the added neutrophils adhered to the stimulated HUVEC at a concentration of LPS of 100 ng/ml. After prolonged stimulation of the HUVEC with LPS (24 hours), there is a significant drop-off in the adhesion of neutrophils to about 35%, but this remains above the basal level of adhesion to unstimulated HUVEC (graph page 222).

Unstimulated HL60 cells exhibit a similar pattern of adhesion to LPS-stimulated HUVEC. An increase in adhesion is detectable after 1 hour of stimulation, and this peaks after 3 to 4 hours at about 40% of the added cells with a concentration of LPS of 100 ng/ml. A detectable increase in adhesion occurs with as little as 0.5 ng/ml of LPS. There is again a decrease in adhesion after 24 hours of stimulation, but in contrast to neutrophils, the number of HL60 cells remaining adherent has almost returned to the basal level (graph page 222). IL-1 stimulation of HUVEC also results in an increase in adhesion of unstimulated HL60, detectable with 0.1 units/ml of IL-1. Approximately 30% of the added HL60 remain adherent to HUVEC stimulated with 5 units/ml of IL-1 (not shown).

Discussion

These experiments show that neutrophils and the related cell line HL60 have a low basal adhesion to cultured HUVEC, which is consistent with the non-adherent state of unstimulated endothelium in vivo. The basis of this low basal adhesion is unclear from the studies that have investigated it with blocking antibodies. One possibility is that it is mediated at least partly by members of the β_2 integrin family. Buchanan et al (1982) found that unstimulated neutrophils from a patient with LAD had normal interactions with unstimulated endothelial monolayers, and Harlan et al (1985b) could detect no difference between neutrophils from normal donors, patients with LAD, and normal neutrophils coated with anti-CD18 antibody, provided the adhesion assay was performed in serum-free medium.

In contrast, another study has shown that neutrophils from a patient with LAD had lower basal adhesion to HUVEC than neutrophils from a normal subject (Dobrina et al, 1989), and two further studies demonstrated that antibodies against CD18 and ICAM-1 significantly decreased the basal adhesion of normal neutrophils (Smith et al, 1988, Lawrence et al, 1990). These results suggest that β_2 integrins on the neutrophils make some contribution to the adhesion of unstimulated neutrophils to unstimulated HUVEC.

A variety of substances act directly on neutrophils to increase their adhesion to cultured endothelial cells, including the phorbol esters used in this thesis. The other substances include formyl methionyl-leucyl-phenylalanine (FMLP), the calcium ionophore A23187, C5a, leukotriene B4 (LT_{B4}), platelet activating factor (PAF), TNF, and leucocyte inhibitory factor (LIF) (Zimmerman and McIntyre, 1988, Tonnesen et al, 1984, Tonnesen et al, 1989, Dobrina et al, 1989, Prieto et al, 1988, Schainberg et al, 1988, Gamble et al, 1985, Lo et al, 1989, Harlan et al, 1985b). The increased adhesion of stimulated neutrophils is mediated by β_2 integrins, based on evidence from a variety of studies. The increase is blocked by antibodies directed against CD18, neutrophils from patients lacking CD18 (ie with severe LAD) do not become more adherent after stimulation (Prieto et al, 1988, Dobrina et al, 1989, Tonnesen et al, 1989, Zimmerman and McIntyre, 1988, Harlan et al, 1985b, Diener et al, 1985), and antibodies against CD18 inhibit stimulated neutrophil adherence in vivo (Arfors et al, 1987).

LFA-1 and Mac-1 make approximately equal contributions to this increased adhesive capacity of stimulated neutrophils, but p150,95 does not

significantly contribute (Lo et al, 1989). In that particular study, antibody against ICAM-1 blocked the LFA-1-dependent component of the increased adhesion but had no effect on the Mac-1-dependent component, which suggests that ICAM-1 is a ligand for LFA-1 on neutrophils but not Mac-1. However, other studies are not in complete agreement with these findings. Prieto et al (1988) found that only antibodies against Mac-1 inhibited PMA-stimulated neutrophil adhesion to HUVEC. In two other studies (Smith et al, 1988, Smith et al, 1989), antibodies against LFA-1 and Mac-1 each inhibited FMLP-stimulated adhesion of neutrophils to cultured HUVEC, and the increased adhesion was completely blocked by antibody against ICAM-1. Mac-1 also mediated the adhesion of FMLP-stimulated neutrophils to purified ICAM-1 monolayers, suggesting that ICAM-1 is a ligand for Mac-1.

Stimulation of the neutrophils increases their surface expression of β_2 integrins, but this increase is not the cause of the augmented adhesion (Vedder and Harlan, 1988a). It appears to be accounted for by the extra avidity of LFA-1 and Mac-1 for their ligands after stimulation (see section 4.1).

HL60 only express low levels of CD18 molecules (Tonnesen et al, 1989), which is consistent with the low basal adhesion and the little increase in adhesion after PMA-stimulation that was observed here. The study of Tonneson et al also found only a small increase in HL60 adhesion after FMLP or LT_{B4} stimulation.

Monocytes were not studied in this thesis, but other investigators have shown that they have a higher basal adhesion to unstimulated cultured HUVEC than other leucocytes (Prieto et al, 1988, Pawlowski et al, 1985). However, there is some disagreement about the mechanisms of monocyte adherence. Te Velde et al (1987) concluded that only antibodies against p150,95 inhibited monocyte adhesion to HUVEC, whereas Mentzer et al (1987) found that only anti-LFA-1 antibodies were inhibitory. Each group used different antibodies and methods which may explain some of the disagreement. Two other studies found that antibodies against CD18 inhibited monocyte adherence, but did not dissect it further with specific α subunit antibodies (Prieto et al, 1988, Wallis et al, 1985). The former study failed to show any increase in monocyte adherence after phorbol ester stimulation, whereas the latter documented a CD18-dependent increase under the same conditions.

Basophils adhere well to cultured HUVEC, and this is increased by the same stimulants that affect neutrophils (Bochner et al, 1988). The basal and stimulated adhesion are both inhibited by antibody against CD18.

The stimulated increase in adhesion of eosinophils is also inhibited by antibodies against CD18 without affecting their basal adhesion (Kimani et al, 1988, Lamas et al, 1988).

In summary, these leucocytes spontaneously adhere to cultured HUVEC in varying amounts by a mechanism that is partly CD18-dependent. A number of substances increase this adhesion by acting on the adhering cell, and this increase depends on CD18 molecules, probably due to greater avidity rather than more receptors.

The studies in this thesis show that stimulating the HUVEC with LPS also increases neutrophil and HL60 cell adhesion, but the response time and kinetics are quite distinct from the increased adhesion resulting from directly stimulating the adhering cells. A number of studies by other investigators have examined the mechanisms of increased leucocyte adhesion following endothelial cell stimulation.

Four distinct mechanisms have been described to explain increased adhesiveness of stimulated endothelial cells for neutrophils. The first is that mediated by GMP-140 (see section 4.1). The second is less well defined and is dependent on the synthesis of platelet-activating factor (PAF) by the endothelial cells (McIntyre et al, 1986, Lewis et al, 1988, Breviario et al, 1988). These two pathways result in a rapid (minutes) increase in adhesion and do not require de novo protein synthesis. The third (CD18-dependent) and fourth (ELAM-1-dependent) pathways require new protein synthesis and take several hours for full effect. Infecting endothelial cells with viruses also increases neutrophil adherence, but the molecular mechanisms are uncertain (MacGregor et al, 1980, Kirkpatrick et al, 1985).

IL-1, TNF, LT, and LPS stimulate cultured endothelial cells to become more adhesive for neutrophils, monocytes, basophils, eosinophils, HL60, and U937 cells (Bevilacqua et al, 1985a, Bevilacqua et al, 1985b, Pohlman et al, 1986, Broudy et al, 1987a, Schleimer and Rutledge, 1986, Thomas et al, 1988, Dunn and Fleming, 1984, Gamble et al, 1985, Bochner et al, 1988, Lamas et al, 1988). IL-1 and TNF stimulation also increases the adhesion of certain tumour cell lines (Dejana et al, 1988c). Schleimer and Rutledge (1986) found that phorbol esters promoted neutrophil adhesion by an effect on the endothelial cells, but that it did not require protein synthesis. This is a little surprising, as phorbol esters stimulate the de novo expression of ELAM-1.

The time course of the increased adhesion of neutrophils and HL60 cells to LPS-stimulated cultured HUVEC that were found in the studies in this thesis are consistent with the results in other studies, and reflect the requirement for

de novo protein synthesis. The decrease in adhesion of neutrophils and HL60 cells after more prolonged stimulation is presumably due to the diminished expression of ELAM-1 by the HUVEC. As HL60 express only low levels of β_2 integrins, their adhesion falls off after 24 hours to barely above their basal adhesion because they can make little if any use of the ICAM-1 that remains upregulated on the LPS-stimulated HUVEC. In contrast, neutrophils have decreased but still significantly more than basal adhesion after prolonged stimulation of the HUVEC. This suggests that the ICAM-1 on the HUVEC makes a significant contribution to their adhesion, by interacting with neutrophil β_2 integrin molecules.

The relative contributions of the CD18 and ELAM-1 pathways to neutrophil adhesion to cytokine-stimulated HUVEC has been examined in several studies. Smith et al (1988) showed that either anti-CD18 antibodies on the neutrophils or anti-ICAM-1 antibodies on the HUVEC inhibited the adhesion of unstimulated neutrophils to IL-1-stimulated HUVEC by about 50%, and that they did not have an additive effect. In a later study (Smith et al, 1989), the same group showed that the CD18 effect was due to CD11a and not CD11b. This is contradicted in another study (Luscinskas et al, 1989) showing that both CD11a and CD11b contribute to the the CD18-dependent portion of the adhesion after 4 hours of stimulation. Antibodies against ELAM-1 had an additional inhibitory effect on adhesion at this time, but after 24 hours when ELAM-1 expression was markedly decreased, they had no effect. After 24 hours of stimulation, CD18-dependent adhesion predominated and included a contribution from CD11c. These findings are essentially in agreement with those of Pohlman et al (1986) and Dobrina et al (1989). The latter also found that neutrophils from a patient with LAD were able to adhere to stimulated HUVEC by a CD18-independent mechanism, presumably to ELAM-1. The anti-inflammatory compound 3-deazoadenosine (Jurgensen et al, 1990) prevents TNF-induced increased HUVEC adhesion for neutrophils and also inhibits the upregulation of ICAM-1. Although ELAM-1 was not examined, it is probable that its upregulation is also blocked.

Neutrophil adhesion to activated endothelium in vivo is followed by transendothelial migration into the extravascular tissues. Techniques have been developed to study neutrophil adhesion to endothelial cell monolayers under flow conditions, followed by migration through the monolayer. Lawrence et al (1990) demonstrated that neither CD18 nor ICAM-1 significantly contribute to neutrophil adhesion to IL-1-stimulated HUVEC under shear stress conditions, but that antibodies to CD18 and ICAM-1 each inhibit the

transendothelial migration of neutrophils that follows the adhesive step. They concluded that neutrophil adhesion to stimulated HUVEC is CD18-independent (and possibly mediated by ELAM-1), but that CD18 is an important mediator of transendothelial migration. This would explain why neutrophils from patients with LAD can adhere to stimulated cultured HUVEC, but are unable to migrate into the tissues in vivo. The transendothelial migration of neutrophils is also CD18- and ICAM-1-dependent in static assays (Smith et al, 1988). The passage of neutrophils through the endothelial monolayer does not automatically follow after adhesion, as only IL-1- or TNF-stimulated HUVEC allow it to occur (Moses et al, 1989). They proposed that the process be divided into an initial anchoring step (which is probably mainly ELAM-1-dependent), followed by an active neutrophil migration (which is CD18- and ICAM-1-dependent).

The in vivo implication of these findings is that only activated endothelium will allow both neutrophil adhesion and transendothelial migration. The activation is the result of cytokines that are produced by the infiltrating inflammatory cells. Chemoattractants for neutrophils by themselves will stimulate adhesion, but extravasation will not follow unless the endothelium is also activated. TNF uniquely has the dual properties of activating neutrophils and the endothelial cells, and may have a crucial role in neutrophil extravasation at sites of acute inflammation.

Mechanisms also exist to decrease neutrophil adhesion to endothelium. Transforming growth factor-β (TGF-β) inhibits basal neutrophil adhesion and decreases the adhesive response of endothelial cells to TNF (Gamble and Vadas 1988b). This only occurs with early passage endothelial cells which suggests the possibility that it is due to an effect on contaminating leucocytes. Lymphokine-containing preparations suppress the adhesion of P815 mastocytoma or Ehrlich ascites cells to endothelium (Cohen et al, 1985). This effect was attributed to tumour migration inhibition factor (TMIF). Cultured HUVEC stimulated with cytokines or LPS also produce an inhibitor of leucocyte adhesion which acts specifically on neutrophils or monocytes (Wheeler et al, 1988). This inhibitor has been identified as interleukin-8, predominantly an extended amino-terminal variant (Gimbrone et al, 1989). Purified IL-8 also protects the endothelial monolayers from neutrophil-mediated damage.

Thus, even in the midst of proinflammatory and proadhesive stimuli, there exists cytokine-mediated mechanisms for decreasing leucocyte adhesion that may exert important controls at sites of inflammation.

4.2 (2) Adhesion of T lymphocytes and lymphoid cell lines to cultured HUVEC

T lymphocytes to be used for studying adhesion to cultured HUVEC were isolated from the peripheral blood of normal donors. The basal adhesion of T lymphocytes to cultured HUVEC varied between assays from 2.5% to 10% of the total cells added, depending on the cell donor and the batch of HUVEC. The T lymphocytic cell line Jurkat and the EBV-transformed B lymphocyte line Mann were chosen to compare their adhesion characteristics with T lymphocytes. Only 1 to 2% of Jurkat cells but 30 to 70% (varying between assays) of Mann cells adhere to unstimulated HUVEC (graphs page 223).

The marked disparity in the basal adhesion of these two cell lines was investigated by measuring their expression of LFA-1, LFA-3, and ICAM-1 by flow cytometric analysis (see page 224). They both express moderate and comparable levels of LFA-3 and ICAM-1, but Jurkat cells have very low basal expression of LFA-1 compared to a moderately high expression by Mann cells. The LFA-1 on the Mann cells is the major determinant of their high basal adhesion, as an antibody against LFA-1 (TS1/22) inhibits it almost completely (> 90%). An antibody against ICAM-1 on the HUVEC inhibits Mann cell basal adhesion by about 40% (graph page 225).

Jurkat and Mann cells also have different homotypic aggregation characteristics, which reflect their contrasting expressions of LFA-1. In assays that were assessed visually, Jurkat cells have little or no spontaneous aggregation in culture, whereas Mann cells, which normally form large aggregates in culture, re-aggregate within 1 hour of manual dispersal. This re-aggregation is mostly (approximately 75%) inhibited by the TS1/22 antibody against LFA-1, mildly (approximately 25%) inhibited by antibody against ICAM-1 (QE2.1B4 - see later), and unaffected by anti-CD2 (TS2/18) or anti-LFA-3 (TS2/9) antibodies. After PMA (25 ng/ml) is added to the cultures, Mann cells aggregate into larger groups within 1 hour. In the presence of antibody against LFA-1, they remain as single cells, and antibody against ICAM-1 inhibits the aggregation by about 50%. PMA has no comparable rapid effect on Jurkat cells, but after 24 hours of PMA stimulation they have aggregated strongly. This late aggregation is unaffected by antibodies against LFA-1, ICAM-1, CD2, or LFA-3.

A rapid (minutes) increase in adhesion to HUVEC occurs if T lymphocytes, Jurkat cells or Mann cells are stimulated with PMA (graphs page 223). At a concentration of 30 ng/ml of PMA, 5 to 10 times as many T lymphocytes (30 to 40% of added cells) and 3 to 5 times as many Jurkat cells adhere to the

unstimulated HUVEC compared to basal adhesion. However, there is only a slight (25%) increase in the adhesion of Mann cells (above their high basal adhesion) after PMA stimulation.

A clear increase in the adhesion of both T lymphocytes and Jurkat cells occurs after IL-1 or LPS stimulation of the HUVEC. It is detectable within 2 hours of stimulation and peaks at about 4 hours, but decreases after 24 hours of stimulation (by 50% for T lymphocytes and by 30% for Jurkat cells). An increase in adhesion is detectable at a concentration of IL-1 of 1 unit/ml and of LPS of 0.5 ng/ml. The effect is still increasing at 5 units/ml of IL-1, and plateaus at 500 to 1000 ng/ml of LPS. The effects of concentrations of LPS ranging from 0.5 to 50 ng/ml are shown on the graphs on page 230. The maximal adhesion of T lymphocytes after LPS stimulation is 2 to 4 times the basal adhesion (10 to 20% of cells). The response of Jurkat cells to LPS stimulation of the HUVEC is more dramatic, and 20 to 30 times the number of basally adherent cells (30 to 40%) remain adherent. Mann cells, which have much higher basal adherence, only exhibit a marginal increase of adhesion to about 70% when the HUVEC are stimulated with LPS. Typical results for each cell type after stimulation of the HUVEC with a set concentration of LPS (100ng/ml) are shown on the graphs on page 226.

Discussion

The adhesion characteristics of these three cell types varies considerably in these studies. The T lymphocytes have intermediate basal adhesion, in between Jurkat cells (low) and Mann cells (high). This suggests that there is a hierarchy of number and/or activation of adhesion molecules on these cells, and shows that Mann cells intrinsically have the most adhesive phenotype. Mann cells are also the only cells that spontaneously form aggregates in culture, which is inhibited by antibody against LFA-1.

Cavender et al (1988) examined the adhesiveness of subpopulations of T lymphocytes, and found that the cells that adhered well to unstimulated HUVEC had larger volumes and expressed more LFA-1 and Mac-1, which suggests that they have a more "activated" phenotype. The baseline adhesion of T lymphocytes is inhibited by anti-CD18 and anti-CD11a antibodies (Mentzer et al, 1986a, Haskard et al, 1986b). Extrapolating from this, it could be deduced that Jurkat cells might express fewer or less avid β_2 integrins and that Mann cells express more to explain their relative adhesion to HUVEC. The greater expression of LFA-1 by Mann cells is confirmed by the flow

cytometric analysis described above, and is a consistent feature of other lymphoblastoid cell lines which spontaneously aggregate (Billaud et al, 1990). The increased expression of LFA-1 appears to be determined by the presence of a latent infection membrane protein (LMP) encoded by the Epstein-Barr virus genome (Wang et al, 1988). The correlation between LFA-1 expression and cellular adhesiveness suggests that the number of integrin molecules on the surface of a cell is an important determinant of the its adhesive phenotype. This does not preclude a contribution to a cell's baseline adhesion characteristics by variations in the avidity of its adhesion receptors.

The rapid increase in T lymphocyte adhesion to HUVEC observed here after phorbol ester stimulation has been well documented in other studies (Haskard et al, 1986a). The studies in this thesis also show that PMA uncovers a significant potential for increased adhesion by Jurkat cells, which is probably due to a similar mechanism as for T lymphocytes. In contrast, Mann cells show little increase in adhesion after PMA stimulation, which may mean that the large number of LFA-1 molecules that they express are already in a highly avid state or conformation.

Phorbol esters also stimulate the homotypic aggregation of lymphocytes (Patarroyo et al, 1983a). This is dependent on the clustering and/or increased avidity of LFA-1 (Patarroyo et al, 1983b), as there is no increased expression of LFA-1 on phorbol ester-stimulated T lymphocytes (Haskard et al, 1986b). Phorbol ester-stimulated adhesion of T lymphocytes to HUVEC is also CD18/CD11a-dependent (Haskard et al, 1986b).

There are several pathways that mediate the binding of T lymphocytes to activated HUVEC, comparable to the situation with neutrophils. One example is the rapid increase in adhesion that occurs after thrombin (Saegusa et al, 1988) or LT_{B4} (Renkonen et al, 1988) stimulation of the HUVEC, which suggests that GMP-140 (or a similar molecule) can support early lymphocyte adhesion. A rapid adhesion pathway dependent on PAF has also been described (Renkonen et al, 1989a).

Another example is the stimulation of HUVEC with γ-IF, which increases T lymphocyte adhesion without affecting neutrophil adhesion (Yu et al, 1985, Hendriks et al, 1989). This increase is detectable after 4 hours of stimulation and maximal after 24 to 48 hours. One study found that it is inhibited by antibodies against HLA-DR (Masuyama et al, 1986).

The increased adhesion of T lymphocytes to HUVEC stimulated with IL-1 or LPS in the studies described in this thesis is comparable to that found in other studies (including the increase following TNF stimulation) (Cavender et

al, 1986, Cavender et al, 1987a, Yu et al, 1986). Cultured dermal microvascular endothelial cells also behave like cultured HUVEC in T lymphocyte adhesion studies (Haskard et al, 1987). The increase peaks after several hours, depends on active metabolism and does not occur at 4°C, and requires new protein synthesis (Cavender et al, 1987a). Antibodies against CD18 and CD11a only weakly inhibit the adhesion of T lymphocytes to IL-1-, TNF-, or LPS-stimulated HUVEC (Haskard et al, 1986b), suggesting that the CD18/CD11a pathway makes little contribution to adhesion to cytokine-stimulated HUVEC. Lymphoblastoid cells from a patient with LAD adhered well to stimulated HUVEC, which also shows that a CD18-independent pathway is the more important mediator of this interaction (Haskard et al, 1989). The same cells, which express less than 10% of the normal level of CD18, did not significantly change their adhesiveness after phorbol ester stimulation.

In the studies described in this thesis, Jurkat cells have very low basal adherence (because they express little LFA-1), but have markedly increased adherence to LPS-stimulated HUVEC, which suggests that this increase is largely CD18-independent. In that particular respect, Jurkat cells behave like the lymphoblastoid cells from patients with LAD. However, they do express sufficient amounts of LFA-1 to respond significantly to phorbol ester stimulation. In contrast, Mann cells have a high level of CD18-dependent adhesion and exhibit little change in their adhesiveness after LPS-stimulation of the HUVEC, suggesting that the CD18-independent pathway of adhesion is less important for these cells.

T lymphocyte and Jurkat cell adhesion decreases after prolonged (24 hours) LPS-stimulation of the HUVEC (not studied in Mann cells). Another group has shown that the IL-1- and TNF-induced adhesiveness of T lymphocytes also drops off after prolonged stimulation (Cavender et al, 1987b). The most likely explanation for this decrease is because ELAM-1 initially makes some contribution to their adhesion to stimulated HUVEC (Bevilacqua et al, 1989, Graber et al, 1990). The ELAM-1 effect falls away with continued stimulation, but other pathway(s) of adhesion remain to sustain adhesion significantly above the basal level.

Antibodies against CD18 and CD11a significantly inhibit the subsequent transmigration of T lymphocytes that occurs following their adhesion to IL-1-stimulated HUVEC monolayers, even though they have little effect on the initial adhesion (similar to neutrophils) (van Epps et al, 1989).

The conclusion from the observations described in this section is that while ELAM-1 and CD18/CD11a separately make some contribution to T lymphocyte adhesion to cytokine-stimulated HUVEC, another mechanism must exist to account for the remaining adhesion that is observed when these two pathways are excluded. It appears that cytokine-stimulated HUVEC mainly support T lymphocyte adhesion by a CD18/ELAM-1-independent pathway, but that subsequent emigration of the cells is dependent on CD18. In allograft rejection, where the endothelium is bathed in cytokines released by infiltrating mononuclear cells, this predicted additional adhesion mechanism is likely to have considerable significance in the adhesion and extravasation of T lymphocytes.

4.3 Production and characterisation of a murine monoclonal antibody against ICAM-1

A strategy was adopted to attempt to develop monoclonal antibodies against HUVEC cell surface molecules that are expressed exclusively or in greater amounts after stimulation by cytokines. The major intention of this work was to develop a monoclonal antibody that is directed against the principal receptor for T lymphocytes expressed by cytokine-stimulated HUVEC (see above).

BALB/c mice were immunised with HUVEC that had been stimulated with either LPS (1000 ng/ml) (two fusions) or recombinant IL-1 (5 units/ml) (1 fusion) for 4 hours. Antibody screening was performed by cellular ELISA against unstimulated and cytokine-stimulated HUVEC. It was anticipated that an antibody against ELAM-1 would only be positive on stimulated HUVEC, whereas one against ICAM-1 would be weakly positive on unstimulated and more strongly on stimulated HUVEC. Antibodies against other endothelial cell "activation molecules" could show either pattern.

Only one supernatant out of the three fusions contained antibody that reacted more strongly with stimulated HUVEC. The particular hybridoma (QE2.1B4) was subcloned three times and the antigen recognised by the antibody characterised in detail. The antibody is of the IgG_{2a} subclass. Immune ascites was produced as described in the Materials and Methods section, and diluted for use in subsequent experiments.

In a cellular ELISA, QE2.1B4 binds moderately well to unstimulated HUVEC, but clearly more to both IL-1- and LPS-stimulated HUVEC. There is a trend to greater binding with increasing concentration of LPS (graph page 227). The fact that it binds to unstimulated cells excludes the possibility that it recognises ELAM-1.

It has not been possible to immunoprecipitate the antigen recognised by QE2.1B4 despite repeated attempts, so there was no structural information to aid its identification. This suggests that the epitope it recognises is destroyed during the immunoprecipitation procedure.

The distribution of the QE2.1B4 antigen in the human kidney was examined by indirect immunoperoxidase staining of sections of normal kidney. The antibody strongly stains the glomerular and intertubular capillaries in the normal human kidney, and weakly stains the intima of arterioles and venules and the parietal epithelial cells lining Bowman's capsule (photograph page

227). This is identical to the staining obtained with a known antibody against ICAM-1 (WEHI-CAM-1; Boyd et al, 1988).

This information strongly suggests that QE2.1B4 recognises an epitope on ICAM-1. This was confirmed by its specific recognition of a stable L-cell transfectant that expresses high levels of human ICAM-1 (performed in Dr. Andrew Boyd's laboratory, Walter and Eliza Hall Institute, Melbourne).

A series of experiments were then performed to determine whether QE2.1B4 binds to a functional determinant on ICAM-1.

QE2.1B4 and WEHI-CAM-1 both cause mild to moderate (25 to 50%) inhibition of the spontaneous aggregation of Mann cells (assessed visually), but neither have a clearcut effect on PMA-induced aggregation of T lymphocytes. The anti-LFA-1 antibody TS1/22 completely inhibits both forms of aggregation.

The two antibodies against ICAM-1 inhibit the two-way mixed lymphocyte culture (MLC) by 60 to 85%, and the effect of QE2.1B4 was concentration dependent. This inhibition approaches but does not reach that of the anti-HLA class II antibody RM5.112 (at least 90% inhibition). The results of a typical experiment are shown on the graph on page 228.

QE2.1B4 also inhibits the mixed lymphocyte-endothelial reaction (MLER) by 40 to 50%, which is comparable to the inhibition with TS1/22 or RM5.112 (see graph page 228).

4.4: Studies using the monoclonal antibody QE2.1B4 to analyse T lymphocyte adhesion to cultured HUVEC

It is concluded from the experiments described above that QE2.1B4 recognises a functional epitope on ICAM-1. The antibody was then used in a series of adhesion assays to study the role of ICAM-1 in the adhesion of T lymphocytes to cultured HUVEC. The concentration of antibody used in these experiments was ascites diluted 1 in 250, which was shown to be a saturating concentration in preliminary experiments.

The low basal adhesion of T lymphocytes to HUVEC is inhibited by TS1/22 by at least 70%, whereas QE2.1B4 inhibits it by about 30% (graph page 229).

The PMA-stimulated increase in adhesion of T lymphocytes is also inhibited by both antibodies, but TS1/22 has a greater effect. At a low concentration of PMA (0.5 ng/ml), TS1/22 almost completely prevents the increase in adhesion, whereas QE2.1B4 inhibits it by about 35%. At higher concentrations of PMA the TS1/22 inhibition is partially overcome, but it continues to be significantly greater than the inhibition obtained with QE2.1B4 (graph page 229).

The effects of QE2.1B4 and TS1/22 on the adhesion of T lymphocytes to LPS-stimulated HUVEC depends on the concentration of LPS. The assays were routinely performed following 4 hours of stimulation with the LPS. At a low concentration (0.5 ng/ml) of LPS, both antibodies almost completely inhibit the increased adhesion of T lymphocytes to the stimulated HUVEC. However, as the concentration of LPS increases, the inhibitory effects of both antibodies decrease, and at 50 ng/ml, neither antibody has any influence on the increased adhesion (graphs page 230).

Discussion

The persistent difference between the inhibitory effects of TS1/22 and QE2.1B4 on unstimulated and stimulated T lymphocyte adhesion to unstimulated HUVEC suggests that an alternative ligand for LFA-1 exists on HUVEC. This would also explain the different effects of the two antibodies on cell aggregation.

The possibility of an alternative ligand for LFA-1 was first suggested when an antibody against LFA-1 but not ICAM-1 inhibited aggregation of the T lymphocyte cell line SKW-3 (Rothlein et al, 1986b). Dustin and Springer

(1988c) concluded that peripheral blood lymphocyte and T lymphoblast (prepared by concanavalin A stimulation) adhesion to unstimulated HUVEC was partly dependent on LFA-1 but completely independent of ICAM-1, suggesting that the LFA-1-dependent portion relied on an alternative ligand on the HUVEC. They also found that an LFA-1-independent component accounted for some of the adhesion of these cells to the HUVEC. This component does not depend on CD2 or LFA-3 as a number of studies have shown that neither molecule plays a role in T lymphocyte adhesion to HUVEC (Haskard et al, 1986b, Mentzer et al, 1986a, Dustin and Springer, 1988c).

There have not been any reports of an antibody that blocks the ICAM-1-independent portion of the LFA-1-dependent pathway. However, an alternative ligand for LFA-1 has been directly cloned and named intercellular adhesion molecule-2 (ICAM-2) (Staunton et al, 1989b). In these experiments, COS cells were transfected with cDNA from endothelial cells, and those expressing ICAM-2 were isolated by adherence of the transfected COS cells to LFA-1-coated Petri dishes in the presence of monoclonal antibody against ICAM-1.

ICAM-2 is an integral membrane protein with two immunoglobulin-like domains, and so is a member of the immunoglobulin superfamily. It is most closely related to the two most amino-terminal domains of ICAM-1 (34% identity). Its mRNA has high basal expression in endothelial cells without being further induced by LPS, and is also found in a wide variety of cells including Ramos and BBN B lymphoblastoid cells, U937, and SKW-3 T lymphoblastoid cells. ICAM-2 is a strong candidate as an alternative ligand for LFA-1, but this has not been proven due to the inability to produce anti-ICAM-2 antibodies. This suggests that access to the ICAM-2 on the cell surface is somehow blocked, preventing antibody formation, or that the ICAM-2 itself is poorly immunogenic.

Some general conclusions can be made from these studies about the mechanisms of T lymphocyte adhesion to unstimulated HUVEC. Basal adhesion is largely mediated by LFA-1 on the T lymphocyte, interacting with ICAM-1 and another ligand, probably ICAM-2, on the HUVEC. The possibility remains that another adhesion pathway(s) accounts for the remaining portion of the basal adhesion. The increase in adhesion after phorbol ester stimulation of the T lymphocytes is also mainly LFA-1-dependent (some studies suggest completely) and relies on ICAM-1 and possibly ICAM-2 on the HUVEC.

The studies described in this thesis show that stimulating HUVEC with low concentrations of LPS increases adhesion of T lymphocytes by a largely LFA-1- and ICAM-1-dependent pathway. However, at higher concentrations of LPS, the increased adhesion is independent of both LFA-1 and ICAM-1, and probably relies on an alternative receptor expressed by the stimulated HUVEC. This is in general agreement with the studies of Dustin and Springer (1988c), except that this group found that there was a LFA-1-dependent component (divided into ICAM-1-dependent and -independent portions) to the adhesion of peripheral blood lymphocytes (which include a significant proportion of B lymphocytes) to HUVEC stimulated with high concentrations of IL-1, TNF, or LPS. They also found that T lymphoblast adhesion to TNF-stimulated HUVEC was completely independent of LFA-1.This is a particulary important finding as this combination of cells is arguably the most relevant to inflammation in vivo. T lymphoblasts from a patient with LAD (with less than 1% of the normal expression of LFA-1) had lower than normal adhesion to unstimulated HUVEC, but still had significantly increased adhesion to cytokine-stimulated HUVEC.

This predicted additional mechanism of adhesion to endothelium that has been exposed to cytokines is potentially very important in the control of lymphocyte accumulation at inflammatory sites in vivo (including allograft rejection). By analogy with the studies on neutrophil adhesion and transendothelial migration (Lawrence et al, 1990), it is postulated that the LFA-1-independent pathway mediates the initial adhesion of T lymphocytes to activated endothelium in vivo, but that extravasation then depends on the LFA-1/ICAM-1 pathway (which is facilitated by the increased avidity of LFA-1 on activated T lymphocytes and the increased ICAM-1 expressed by the stimulated endothelium). Further studies are required to elucidate the mechanisms of T lymphocyte adhesion to HUVEC monolayers under flow conditions, and the subsequent transendothelial migration.

Unfortunately, while the strategy used in these studies resulted in the production of a monoclonal antibody against ICAM-1, it has not been successful in producing a monoclonal antibody that binds to the predicted "new" receptor on HUVEC.

A number of other investigators have also attempted to characterise this receptor. One possibility is recognised by the monoclonal antibody 4D10, which binds to an endothelial antigen only expressed in tissue sections during acute inflammatory reactions (Goerdt et al, 1987). The antigen has a molecular weight of 81 kD and is induced on cultured HUVEC by LPS, TNF,

IL-1, and phorbol esters. In contrast to ELAM-1 it is still present after 24 hours of stimulation, and it is not found on normal or chronic inflammatory tissues, which distinguishes it from ICAM-1. The antibody has no effects on lymphocyte adhesion in vitro, and so its relationship to the endothelial activation antigen discussed above is uncertain.

Other groups have been more successful in this search, in that they have found an inducible endothelial antigen involved in lymphocyte adhesion, which appears to correspond to the predicted receptor on cytokine-stimulated HUVEC.

Inducible cell adhesion molecule 110 (INCAM-110) was originally identified as a receptor for the adhesion of melanoma cell lines to stimulated HUVEC (Rice and Bevilacqua, 1989). This process has comparable kinetics to T lymphocyte adhesion and is not inhibited by antibodies against ICAM-1 or ELAM-1 (Rice et al, 1988). INCAM-110 is detectable in low amounts on resting HUVEC, but is increased by TNF stimulation. The monoclonal antibody E1/6 immunoprecipitates 110 kD and 95 kD species from biosynthetically labelled cell extracts, which both revert to a 76 kD precursor after removal of N-linked carbohydrates. The antibody inhibits the adhesion of melanoma cells, lymphocytes, and monocytes (but not neutrophils) to cytokine-stimulated HUVEC, and has additive effects with the anti-LFA-1 antibody TS1/22, showing that it does not depend on CD11a/CD18 for adhesion (Rice and Bevilacqua, 1989, Rice et al, 1990). The combination of antibodies against INCAM-110 and LFA-1 blocks most of the adhesion of lymphocytes to IL-1-stimulated HUVEC, which suggests that INCAM-110 accounts for at least part of the adhesion pathway predicted above. The antigen is not found on the endothelium of normal human skin, but is strongly expressed in small vessels (predominantly venules) associated with florid perivascular inflammatory infiltration in a delayed hypersensitivity reaction to tuberculin, in vascular endothelium in a cutaneous insect bite reaction, and focally in venular endothelium in peripheral lymph node and tonsil. However, it does not mark HEV selectively (Rice et al, 1990). The INCAM-110 antigen is also expressed by follicular dendritic cells in lymphoid germinal centres (Freedman et al, 1990), where it supports the adhesion of activated B lymphocytes and B cell lines.

The inducible endothelial cell antigen called vascular cell adhesion molecule 1 (VCAM-1) (Osborn et al, 1989) has now been shown to be identical to INCAM-110 (RIce et al, 1990). VCAM-1 was directly cloned by transfecting COS cells with cDNA from IL-1-stimulated HUVEC, and selecting

those COS cells that bound lymphoid cells. The VCAM-1 mRNA is barely detectable in unstimulated HUVEC, but is greatly increased after TNF stimulation. The increase is sustained for at least 72 hours. The mature protein is predicted from the cDNA sequence to have a 582 amino acid extracellular domain, 22 amino acid transmembrane region, and a 19 residue cytoplasmic tail, with a molecular weight of 69 kD. Glycosylation of the 6 potential N-glycosylation sites gives a predicted molecular weight of 90 kD. VCAM-1 is a member of the immunoglobulin superfamily and contains 6 immunoglobulin-like domains.

A monoclonal antibody (4B9), that was produced against TNF-stimulated HUVEC, inhibits the LFA-1-independent adhesion of T lymphocytes to stimulated endothelium and recognises a functional epitope on VCAM-1 (Carlos et al, 1990). The inhibitory effect on T lymphocyte adhesion was additive to that of an antibody against CD18. 4B9 also partially inhibited the adhesion of lymphocytes to unstimulated HUVEC, suggesting that the low levels of VCAM-1 present normally on HUVEC contribute to the basal adhesion of lymphocytes. This inhibition was also additive to that of the anti-CD18 antibody.

Wellicome et al (1990) described a 95 kD antigen, recognised by the antibody 1.4C3, that is minimally expressed by unstimulated HUVEC, but is induced by IL-1, TNF, or LPS. It is also induced by IL-4 (unlike ICAM-1 or ELAM-1), which in turn has synergy with IL-1 and TNF (Thornhill et al, 1990). The antigen is distinct from ELAM-1 and ICAM-1, and has the characteristics of VCAM-1, but has no effect on the adhesion of T lymphocytes to cytokine-stimulated HUVEC ie it does not bind to a functional epitope. The same group has demonstrated that IL-4 stimulation of HUVEC increases lymphocyte adhesion by an LFA-1/ICAM-1-independent mechanism (Thornhill et al, 1990), and this is probably dependent on VCAM-1.

Another group analysed the new membrane proteins synthesised by cultured HUVEC within 6 hours of stimulation with IL-1 (19 out of over 600 proteins), and prepared 2 monoclonal antibodies (1E7 and 2G7) which recognise VCAM-1 (Graber et al, 1990). The antibody 2G7 inhibits the binding of T lymphocytes but not granulocytes to IL-1-stimulated HUVEC.

This LFA-1-independent adhesion pathway has been further characterised by the finding that the receptor for VCAM-1 on lymphoid cells is VLA-4 (Elices et al, 1990). This is another example of interaction between members of the immunoglobulin and integrin families. K562 cells transfected with cDNA of the VLA-4 α subunit specifically adhere to VCAM-1, and this is

inhibited by antibodies against VLA-4. The same antibodies block the adhesion of the lymphoid cells Ramos and HPB-ALL to TNF-stimulated HUVEC and to VCAM-1 expressed in COS cells, as does goat antiserum against the β_1 chain. Monoclonal antibodies directed against the VLA-4 α chain, the β_1 chain, and VCAM-1 all inhibit the adhesion of B lymphoblastoid cells from a patient with LAD (ie CD11/CD18-deficient) to unstimulated HUVEC and TNF-stimulated HUVEC (Schwartz et al, 1990), and inhibit the adhesion of activated B lymphocytes and B cell lines to lymphoid follicular dendritic cells (Freedman et al, 1990). The site of attachment to VCAM-1 on VLA-4 is separate from that which binds to the heparin II binding region of fibronectin. At sites of inflammation, VLA-4 could potentially bind to VCAM-1 expressed by the endothelium as well as fibronectin that is produced by the inflamed cells.

Antibodies against VLA-4 α chain inhibit the attachment of certain cell lines to Peyer's patch HEV and of human lymphocytes to appendix HEV (see section 3). This may be due to inhibition of the interaction between LPAM-1 ($\alpha_4\beta_P$) and its unknown ligand on the HEV. It is likely that VLA-4 is the lymphocyte and monocyte receptor for VCAM-1 expressed by inflamed endothelium.

It is quite possible that VCAM-1/INCAM-110 is expressed by the endothelium during allograft rejection, and that it corresponds to one of the vascular addressins (eg MECA 325, MECA 32). There is also the exciting possibility of using antibodies against VLA-4 and/or VCAM-1/INCAM-110 as therapeutic tools to block the adhesion of lymphocytes to the endothelium specifically at sites of inflammation.

4.5: Studies of the Tissue distribution of ICAM-1

4.5: (1) ICAM-1 in the normal kidney and during renal allograft rejection

This study was undertaken to investigate any changes in expression of ICAM-1 that occur during the alloimmune response to a transplanted kidney. Tissue sections from normal kidneys and from renal allografts undergoing rejection were stained with antibody against ICAM-1 (WEHI-CAM-1) using the indirect immunoperoxidase technique described in Materials and Methods. The sections of normal kidney were cut from the macroscopically uninvolved portions of 2 kidneys removed surgically for localised renal cell carcinoma, and from 11 kidneys just prior to their use as transplants.

ICAM-1 is moderately strongly expressed on the endothelium of glomerular and intertubular capillaries, weakly on the intima of arterioles, venules, and larger vessels, and moderately on the parietal epithelial cells that line Bowman's capsule (see section 4.3 and photograph page 227). The tubules were negative apart from 2 of the pre-transplant kidneys which had some weak staining of the luminal aspect of the tubular cytoplasm.

The recipients of the 11 transplanted kidneys that had been biopsied subsequently had 12 biopsies taken at a later date during episodes of allograft dysfunction (ie one patient had 2), and the expression of ICAM-1 was compared with the pre-transplant biopsies. Both sets of biopsies were also stained with the anti-HLA class II antibody RM5.112 (see section 2.2). The 12 biopsies taken during allograft dysfunction were independently assessed for histological evidence of rejection. Eight biopsies had prominent peritubular infiltration of mononuclear cells consistent with rejection, and the remaining biopsies showed no evidence of rejection. In those cases the dysfunction was caused by cyclosporine nephrotoxicity.

There was a clear increase in the glomerular capillary expression of both ICAM-1 (photograph page 231) and HLA class II during rejection (see section 2.2 and photograph page 67), and this was not observed in the non-rejecting biopsies. The intertubular capillaries are difficult to assess as their architecture is obscured by the cellular infiltrate and by the damage from the rejection process.

There were also changes in the tubular expression of ICAM-1 during rejection. ICAM-1 was expressed on the tubular cytoplasm of 7 out of the 8 rejecting biopsies (photograph page 231), and the same 7 biopsies had tubular HLA class II expression (see section 2.2). Neither ICAM-1 nor HLA

class II were present on the tubules of any of the non-rejecting biopsies. The 2 kidneys which expressed tubular ICAM-1 before transplantation were in the group of non-rejecting biopsies, and in those biopsies the tubular ICAM-1 was no longer present (summarised table 1, page 72).

The tubular expression of ICAM-1 and HLA class II was then examined in a series of 23 randomly selected biopsies from other renal transplants (summarised table 2, page 72). These biopsies were not paired and so it was not possible to directly compare rejecting and non-rejecting biopsies from the same patient.

Seven biopsies had been taken within 60 minutes of re-vascularisation of the allograft. All were morphologically normal and did not show any signs of hyperacute rejection. Detectable ICAM-1 and HLA class II was found on the tubules in 3 of these biopsies, but the other 4 were free of both.

Eight biopsies were diagnostic of acute rejection. Tubular HLA class II was present in all of them and ICAM-1 in 7.

The remaining 8 biopsies showed no evidence of acute rejection. However, 4 showed tubular HLA class II expression, and 2 of those 4 also had tubular ICAM-1.

Discussion

ICAM-1 is normally present on all of the endothelium of the human kidney. It is increased on glomerular capillaries during acute rejection, which is probably due to the cytokines that are produced by the activated cells infiltrating the graft. This is comparable to the increased ICAM-1 expressed by cultured HUVEC after IL-1, TNF, LT, or γ-IF stimulation.

As has been discussed in section 2, the endothelium has a central role in the rejection of a vascularised allograft. It is a target for allorecognition, is capable of presenting alloantigen, and modulates leucocyte adhesion and extravasation. The increased expression of ICAM-1 would facilitate each of these processes.

Additional ICAM-1 should increase the stability of the interaction between the allospecific T lymphocytes and the foreign HLA present on the endothelium, and stimulate the T lymphocytes through the LFA-1 molecules they express. This will increase the target potential of the endothelium (as will its increased expression of HLA class I and class II [see section 2.2]) and contribute to its antigen-presenting capabilities. ICAM-1 also acts as a receptor for the adhesion and extravasation of both allospecific and non-

allospecific leucocytes that accompanies the inflammatory process. The transendothelial migration may be the step that most depends on the upregulation of ICAM-1.

The tubules are often severely damaged early in the rejection of a renal allograft, and they are usually surrounded by a pronounced cellular infiltrate. The increased tubular HLA class I and de novo HLA class II and ICAM-1 conveniently accounts for this particular sensitivity, by making the tubules better targets for allorecognition and possibly giving them the ability to present antigen to sensitised T lymphocytes. The tubular expression of foreign HLA class II is by itself insufficient to initiate rejection (see section 2), but it is expected that it will amplify a pre-existing response. The accompanying ICAM-1 expression will facilitate this sensitisation and allow specific effector cells to adhere to the "activated" tubules. It is again probable that cytokines released by the infitrating cells are the stimulators of this upregulation.

The tubular expression of ICAM-1 during rejection has been reported in another study (Bishop et al, 1989a). They also studied its expression on cultured renal tubular cells, and found it in low amounts basally which increased slightly after treatment with cytokines. ICAM-1 expression is also increased on cultured thyroid cells stimulated with γ-IF, IL-1, or TNF, and on thyroid follicular cells in areas of lymphocytic infiltration in patients with Graves' disease and Hashimoto's thyroiditis (Weetman et al, 1989).

The appearance of ICAM-1 and HLA class II on the tubules can both be markers of rejection. It is unusual for one to be present without the other, or for them to be absent during rejection. However, ICAM-1 is also expressed by the tubules in a number of non-rejecting kidneys, which means that it is not a reliable marker by itself. The expression is usually stronger in the rejection biopsies, but such a subjective assessment is not ideal for diagnosing acute rejection. The concurrent appearance of HLA class II improves the specificity slightly, but it still will not realistically replace histopathological assessment as the primary method for diagnosing rejection.

It is not clear why some of the non-rejecting kidneys expressed tubular ICAM-1. It may be basally expressed in some kidneys, although it was not found in later biopsies from 2 of those patients. New transplants can suffer some degree of reperfusion injury following re-vascularisation, and the consequent inflammation may account for the tubular ICAM-1 in 3 of the 7 biopsies taken within 60 minutes of the re-establishment of blood flow. The most appealing explanation for the tubular ICAM-1 observed in 4 biopsies without rejection is a subtle degree of inflammation that was not regarded as

diagnostic of acute rejection. Two of those patients were diagnosed as having cyclosporine nephrotoxicity, one had acute tubular necrosis, and the other had incipient total infarction following vascular thrombosis. In these cases a small number of infiltrating cells may have released sufficient cytokines to upregulate the ICAM-1.

4.5 (2) ICAM-1 and ELAM-1 in inflammatory vasculitis involving skeletal muscle and peripheral nerves

The specimens of skeletal muscle and peripheral nerve that were stained with antibodies against HLA class I and class II (see section 2.3) were also stained with antibodies against ICAM-1 (WEHI-CAM-1) and ELAM-1 (1.2B6) (Wellicome et al, 1990).

ICAM-1 is weakly expressed on the endothelium of all vessels in the muscle and nerve specimens without inflammation (photographs pages 231 and 232). ELAM-1 is absent in all of these sections.

One patient had muscle biopsies taken both during remission and during an acute exacerbation of inflammatory vasculitis. In the latter biopsy there was an intense inflammatory infiltrate around the blood vessels, which was largely composed of macrophages and lymphocytes, plus a few neutrophils. Here the endothelial expression of ICAM-1 was clearly increased (photograph page 232), and ELAM-1 appeared on the intima of a number of medium to large vessels (photograph page 233).

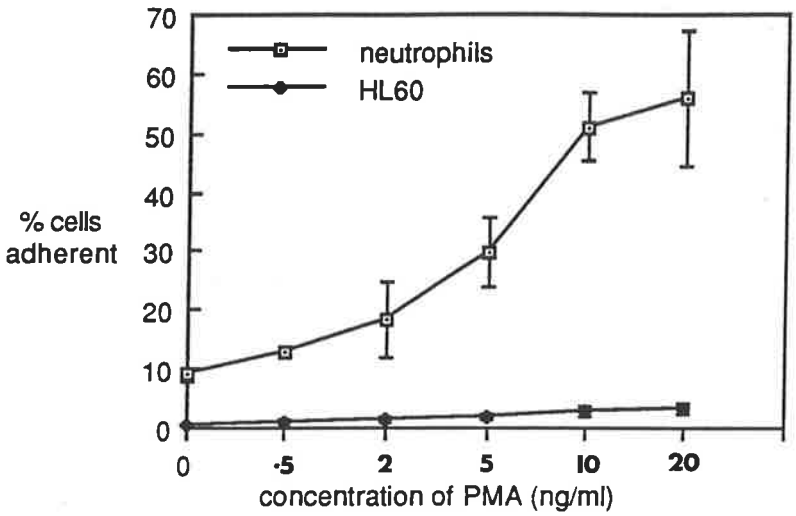
The other biopsies of inflamed muscle and nerve also showed increased endothelial ICAM-1(photograph page 233), but ELAM-1 was only found in the one specimen. ICAM-1 was not expressed by other cells, apart from infiltrating leucocytes.

Discussion

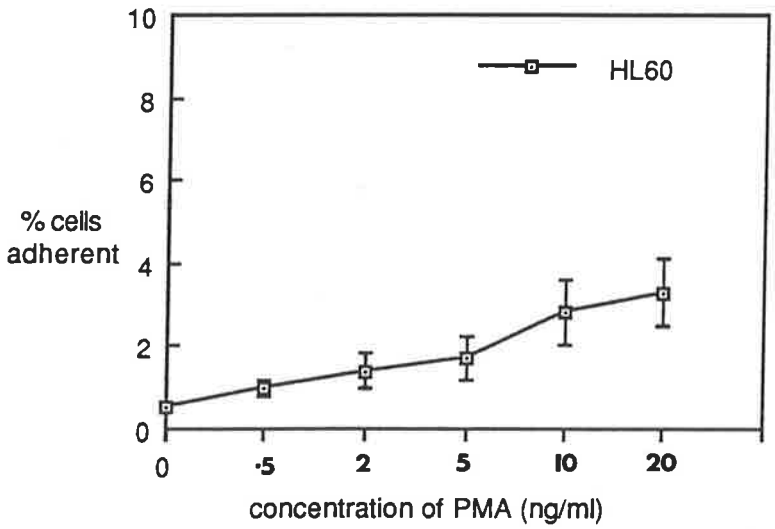
The increased endothelial expression of ICAM-1 in inflammatory vasculitis parallels the increase in HLA class I and class II antigens that was discussed in section 2, and is similar to that observed during renal allograft rejection. This appears to be a general phenomenon on endothelium at sites of inflammation.

There are two possible reasons why ELAM-1 appeared in only one biopsy. It is only transiently expressed by cultured HUVEC in vitro and probably often missed in vivo, and this particular biopsy had the most florid

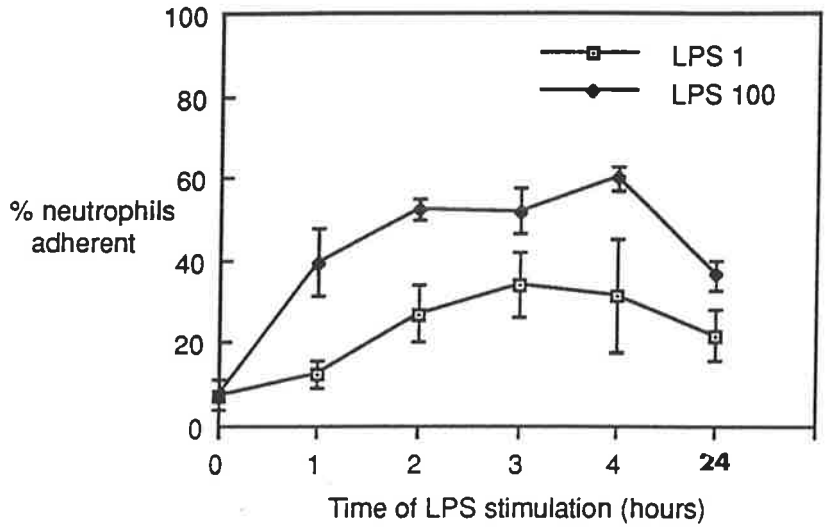
inflammation, which included some neutrophils. The infiltration around the vasculitic vessels is predominantly lymphocytic and monocytic, and ICAM-1 and possibly VCAM-1 are likely to be the most important molecules involved in its accumulation.



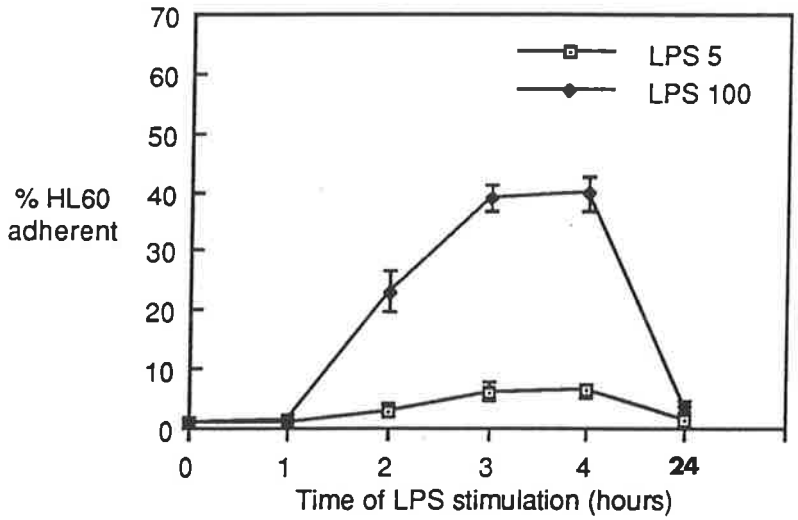
Adhesion of unstimulated and PMA-stimulated neutrophils and HL60 cells to unstimulated HUVEC



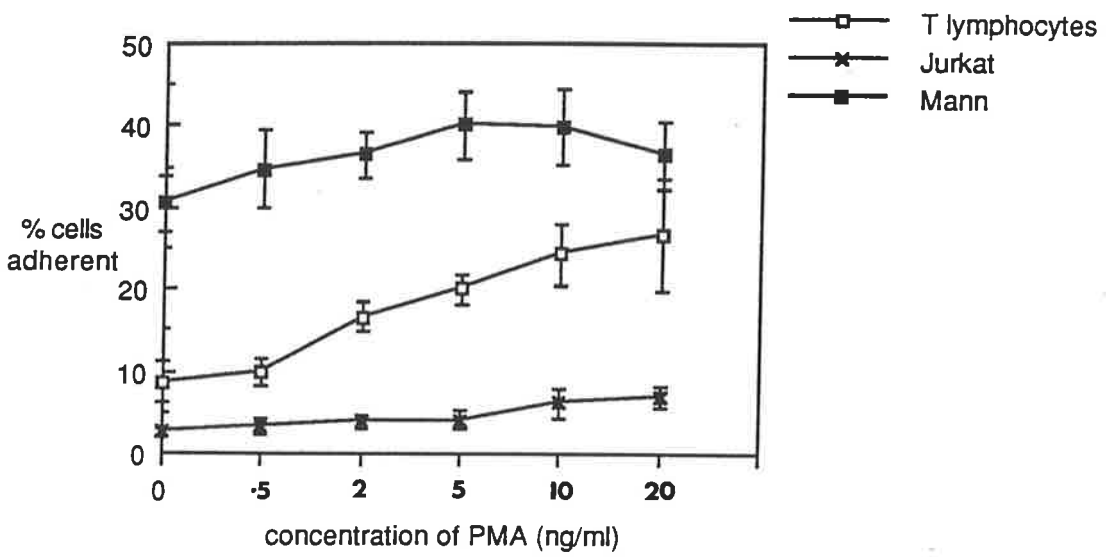
Adhesion of unstimulated and PMA-stimulated HL60 cells to unstimulated HUVEC (using the same data as the graph above but with a different scale on the Y axis)



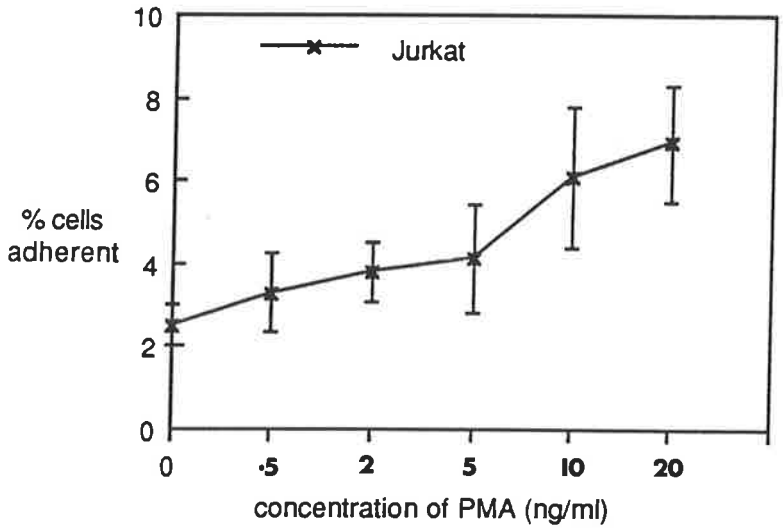
Adhesion of unstimulated neutrophils to HUVEC stimulated with two different concentrations of LPS (1 ng/ml and 100 ng/ml) for increasing periods of time



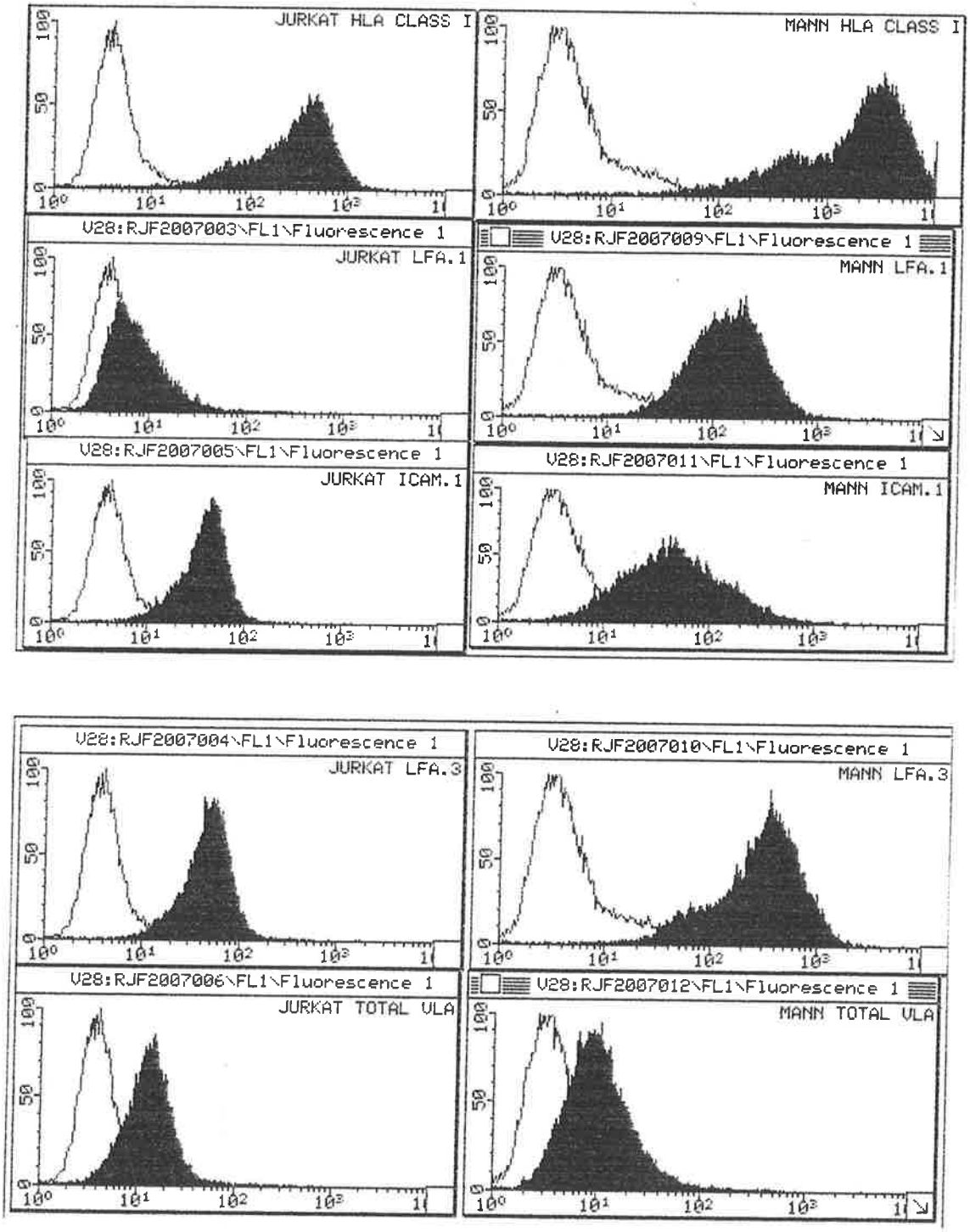
Adhesion of unstimulated HL60 cells to HUVEC stimulated with two different concentrations of LPS (5 ng/ml and 100 ng/ml) for increasing periods of time



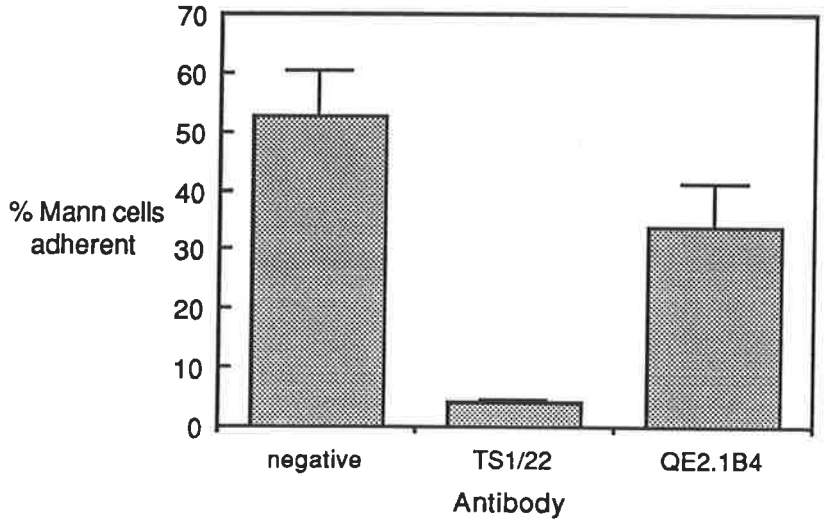
Adhesion of unstimulated and PMA-stimulated T lymphocytes, Jurkat, and Mann cells to unstimulated HUVEC



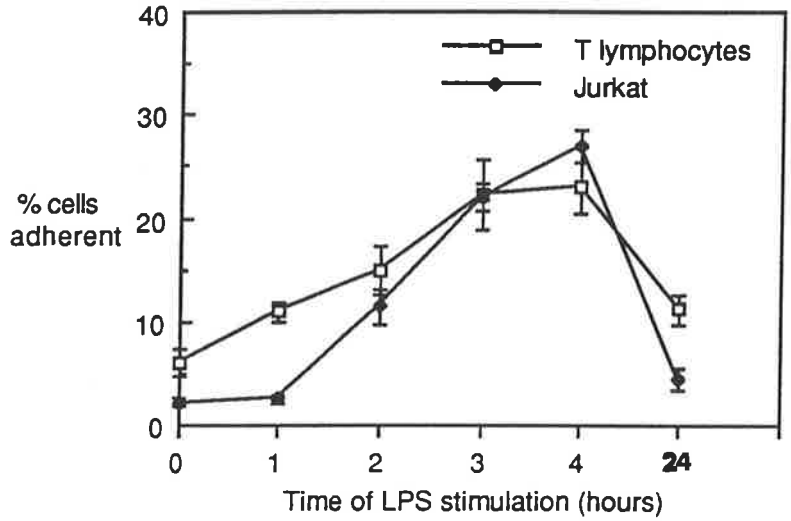
Adhesion of unstimulated and PMA-stimulated Jurkat cells to unstimulated HUVEC (using the same data as the graph above with a different scale on the Y axis)



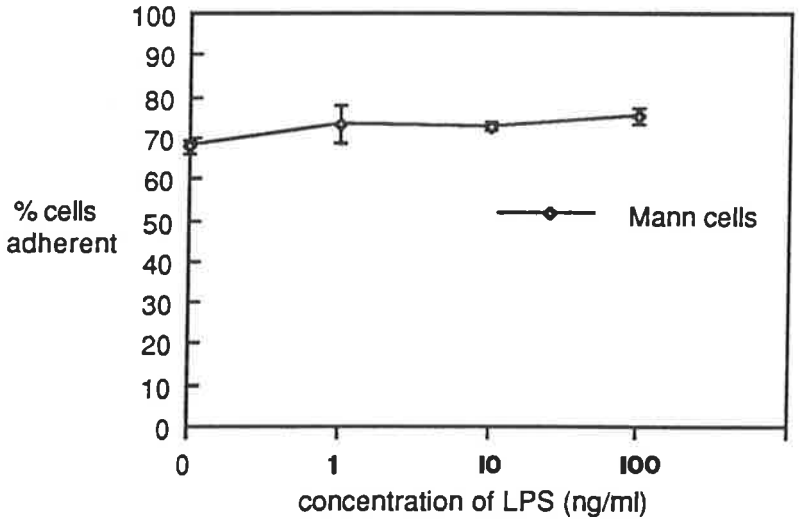
Flow cytometric analysis of the expression of HLA class I and adhesion molecules by Jurkat and Mann cells



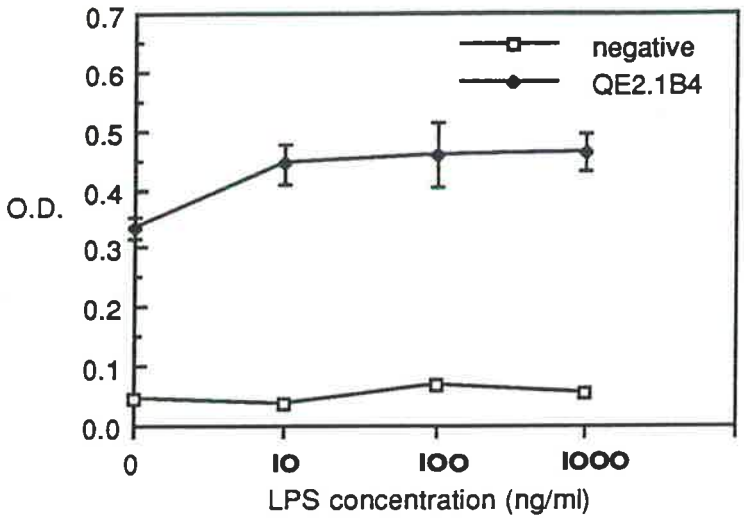
Inhibitory effects of antibodies against LFA-1 (TS1/22) and ICAM-1 (QE2.1B4) on the adhesion of unstimulated Mann cells to unstimulated HUVEC



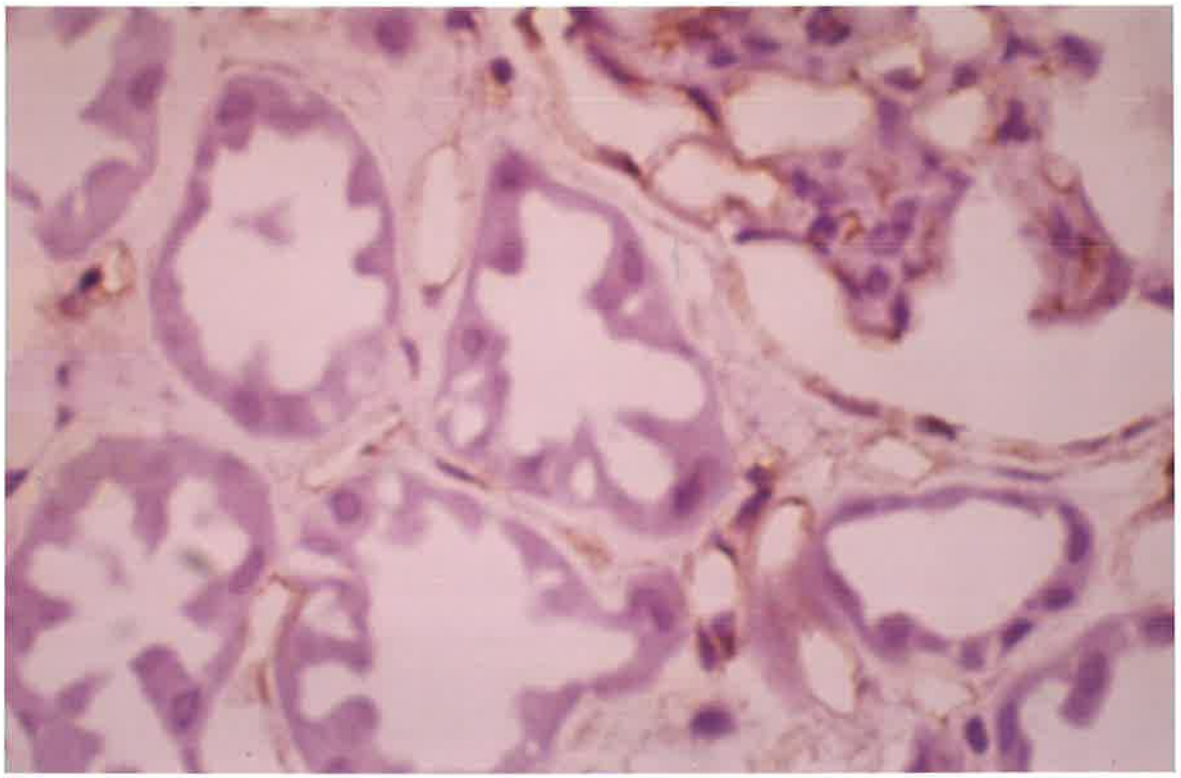
Adhesion of unstimulated T lymphocytes and Jurkat cells to HUVEC stimulated with LPS (100 ng/ml) for increasing periods of time



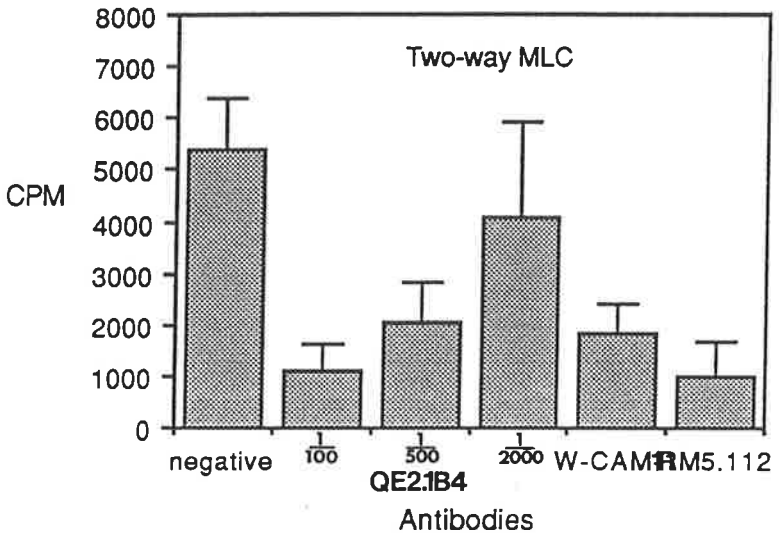
Adhesion of unstimulated Mann cells to HUVEC stimulated with increasing concentrations of LPS



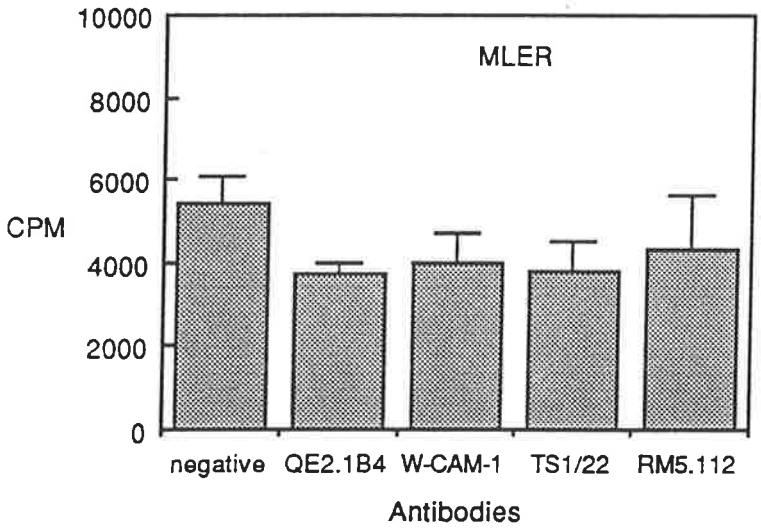
Expression of the QE2.1B4 antigen by cultured HUVEC stimulated with increasing concentrations of LPS (measured by cellular ELISA - see materials and methods)



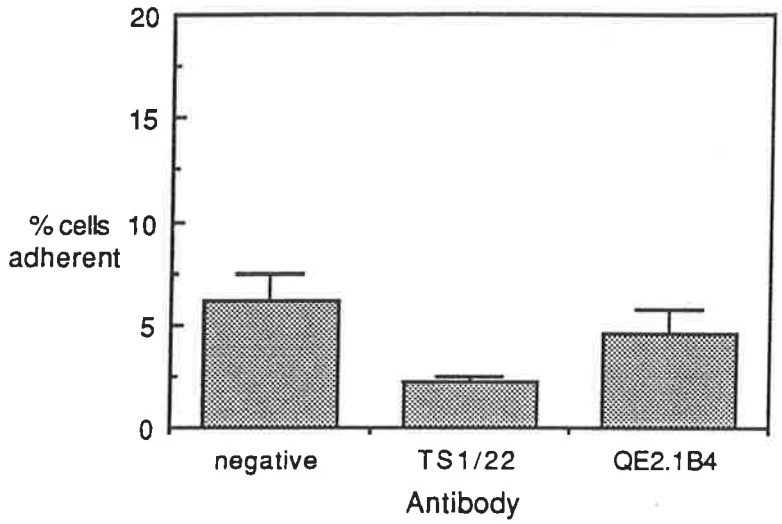
Expression of the QE2.1B4 antigen (ICAM-1) in normal human kidney (indirect immunoperoxidase, x400)



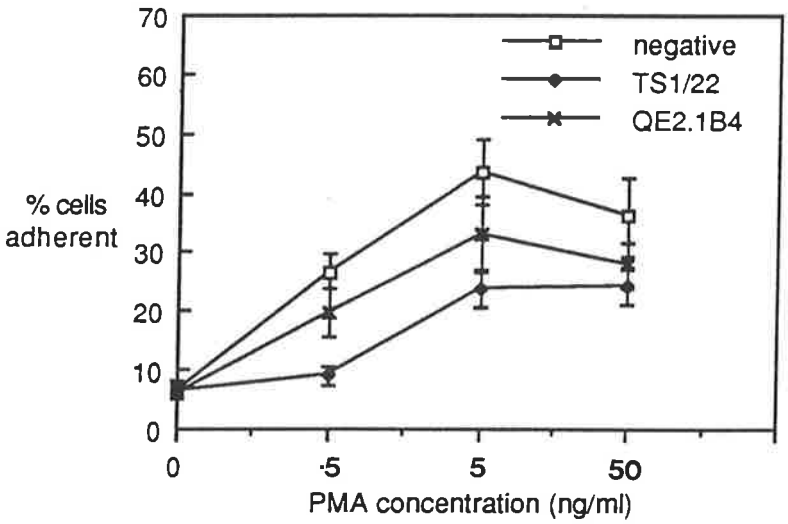
Inhibitory effects of antibodies against ICAM-1 (QE2.1B4, serial dilutions, and WEHI-CAM-1) and HLA class II (RM5.112) on two-way MLC



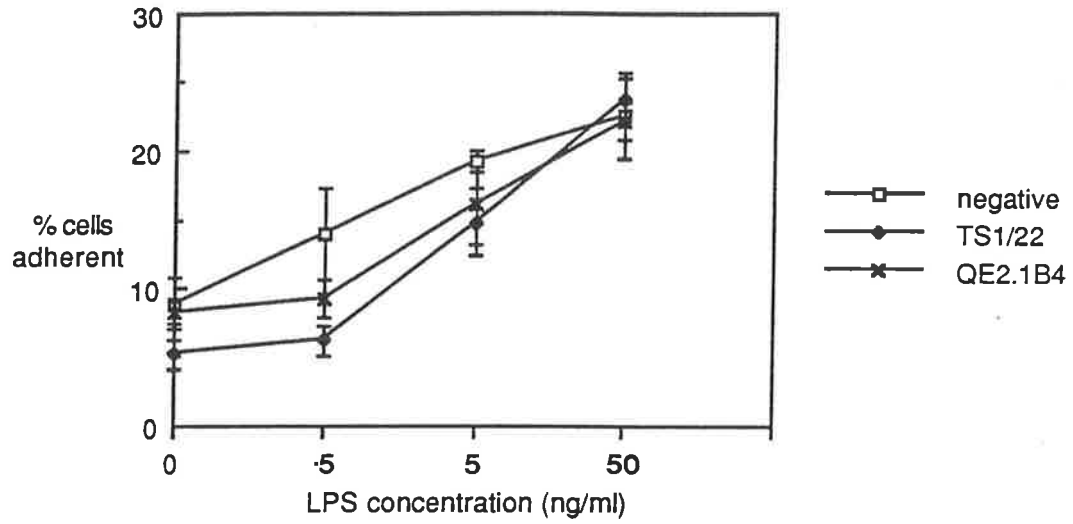
Inhibitory effects of antibodies against ICAM-1 (QE2.1B4 and WEHI-CAM-1), LFA-1 (TS1/22), and HLA class II (RM5.112) on MLER

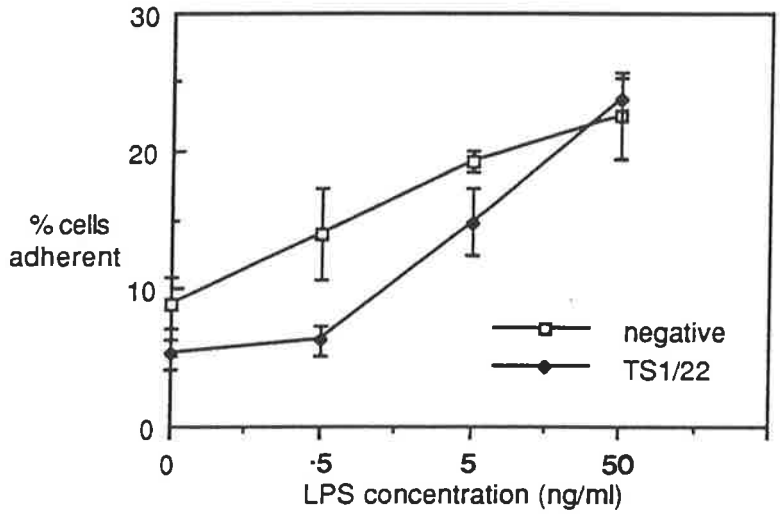


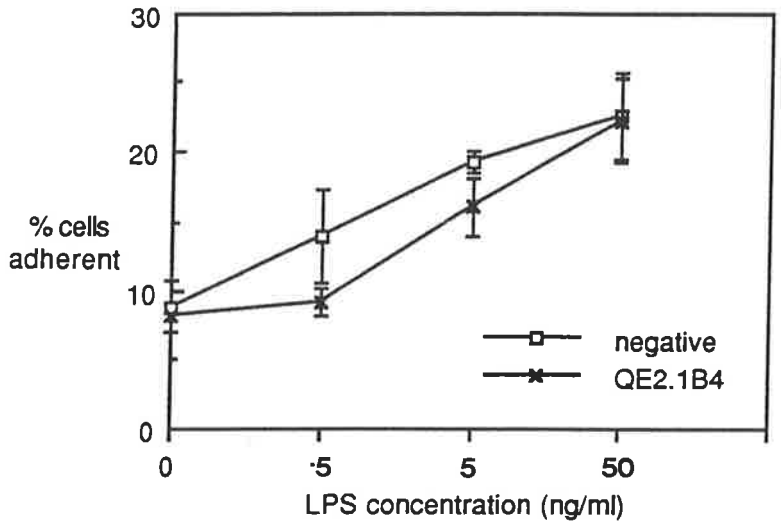
Inhibitory effects of antibodies against LFA-1 (TS1/22) and ICAM-1 (QE2.1B4) on adhesion of unstimulated T lymphocytes to unstimulated HUVEC



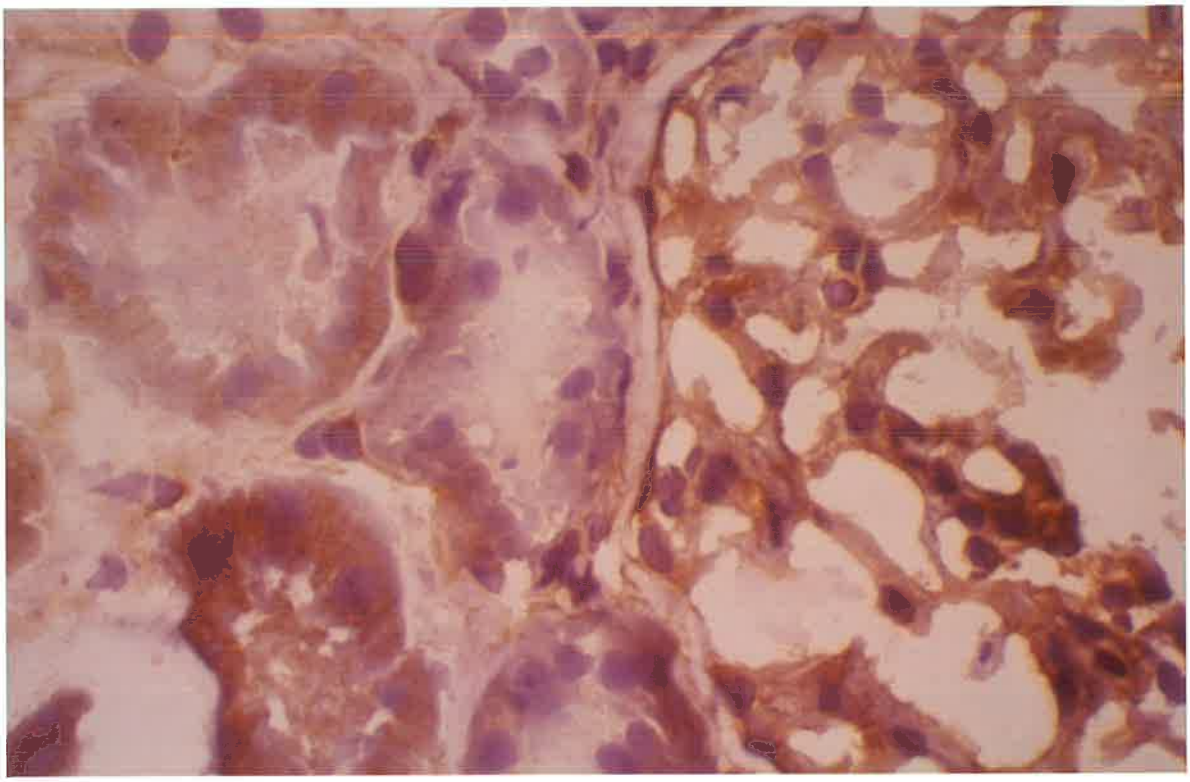
Inhibitory effects of antibodies against LFA-1 (TS1/22) and ICAM-1 (QE2.1B4) on adhesion of PMA-stimulated T lymphocytes to unstimulated HUVEC



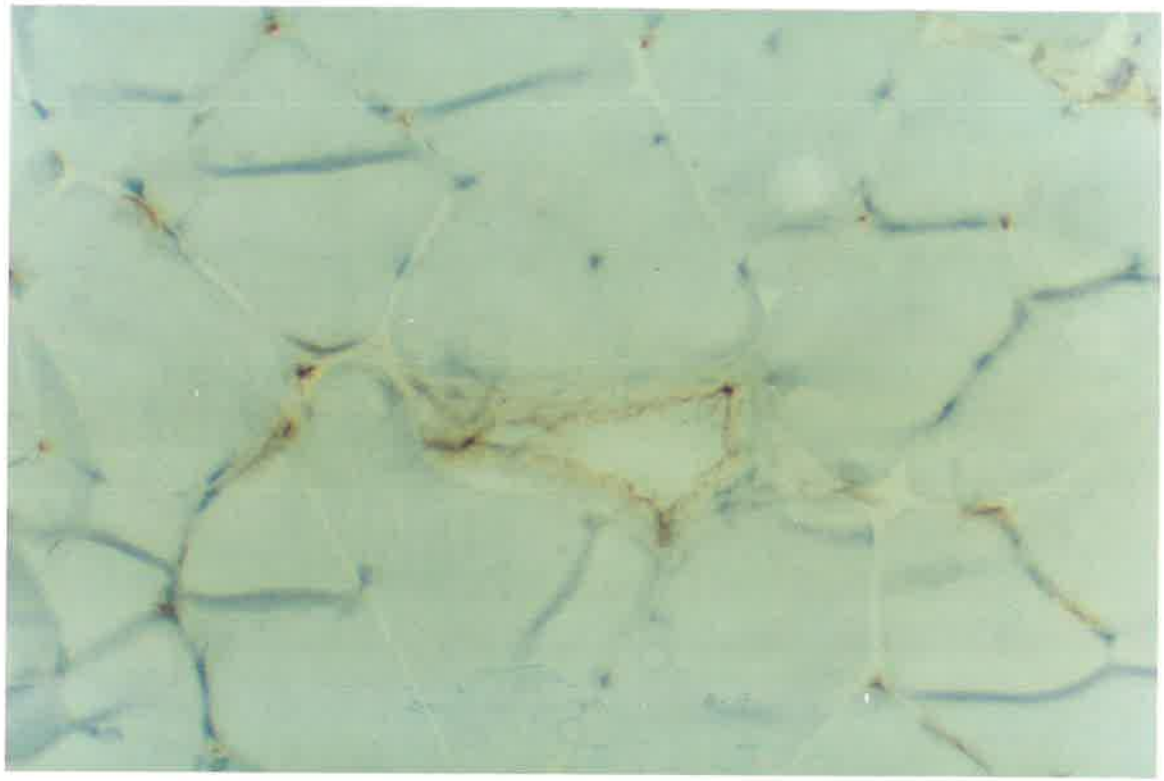




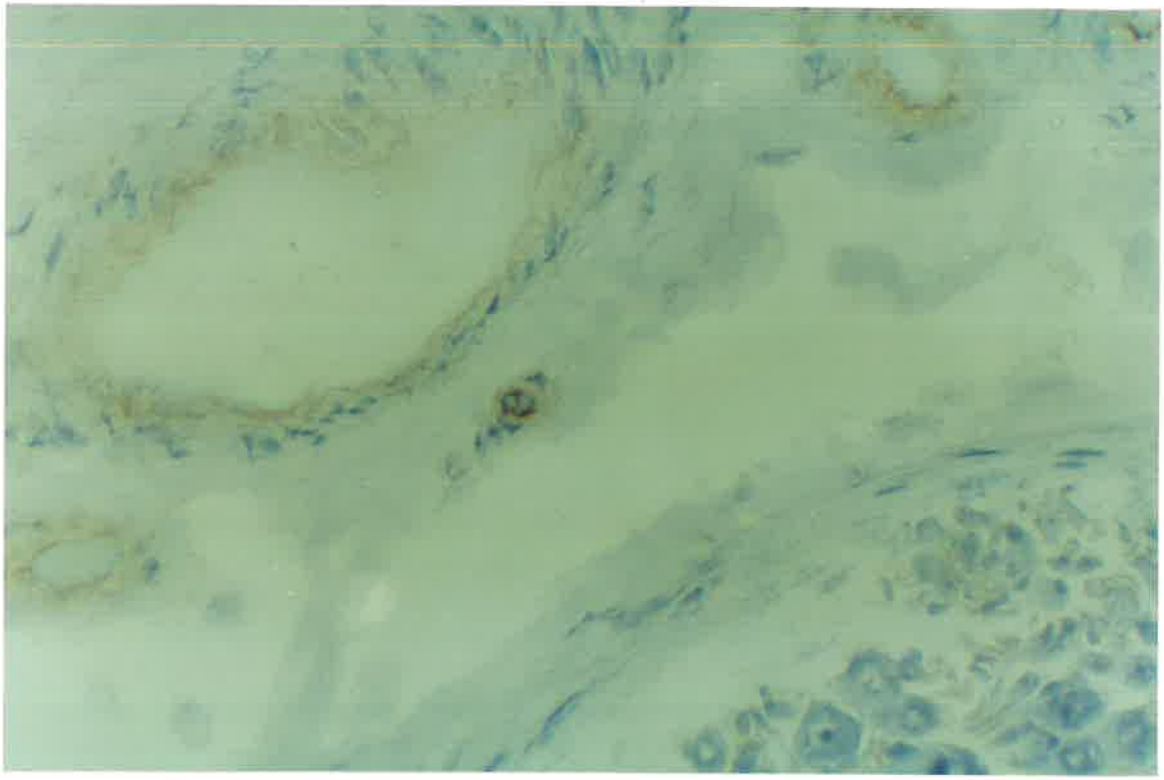
Antibody inhibition of T lymphocyte adhesion to LPS-stimulated HUVEC



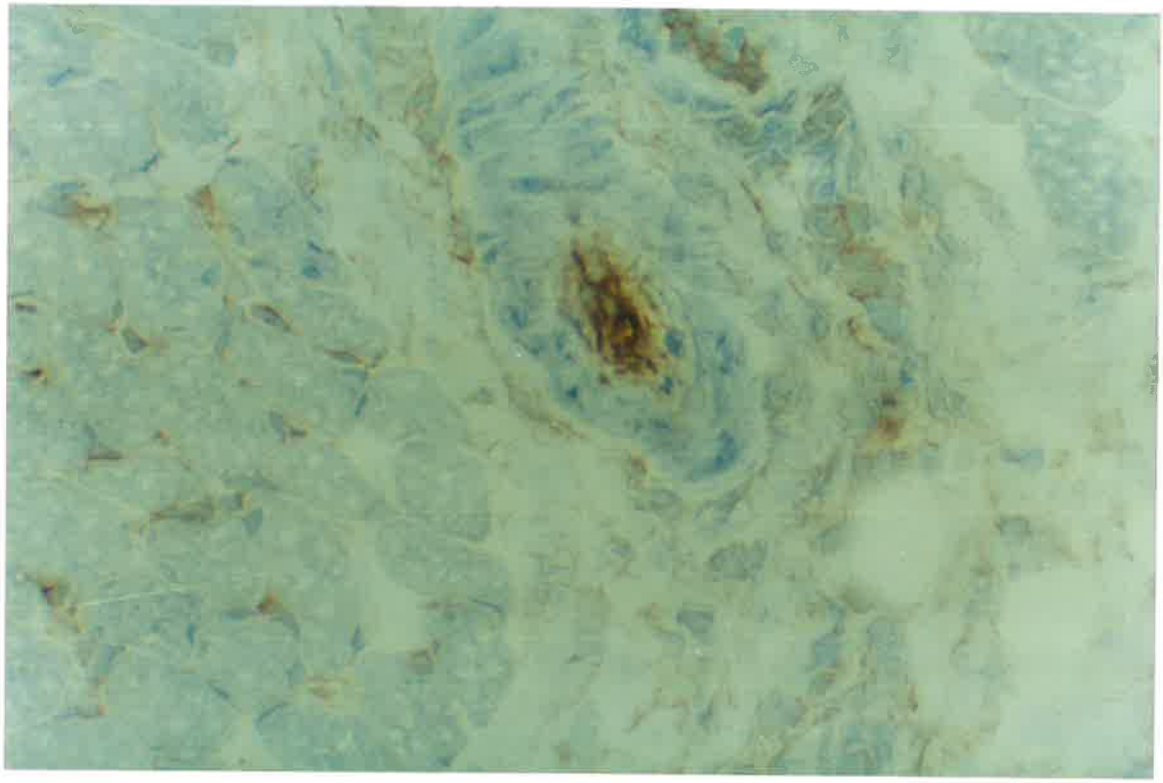
Expression of ICAM-1 (WEHI-CAM-1) on the glomerular capillaries and tubules of a rejecting renal allograft (indirect immunoperoxidase, x400)



Expression of ICAM-1 on normal skeletal muscle (indirect immunoperoxidase, x200)

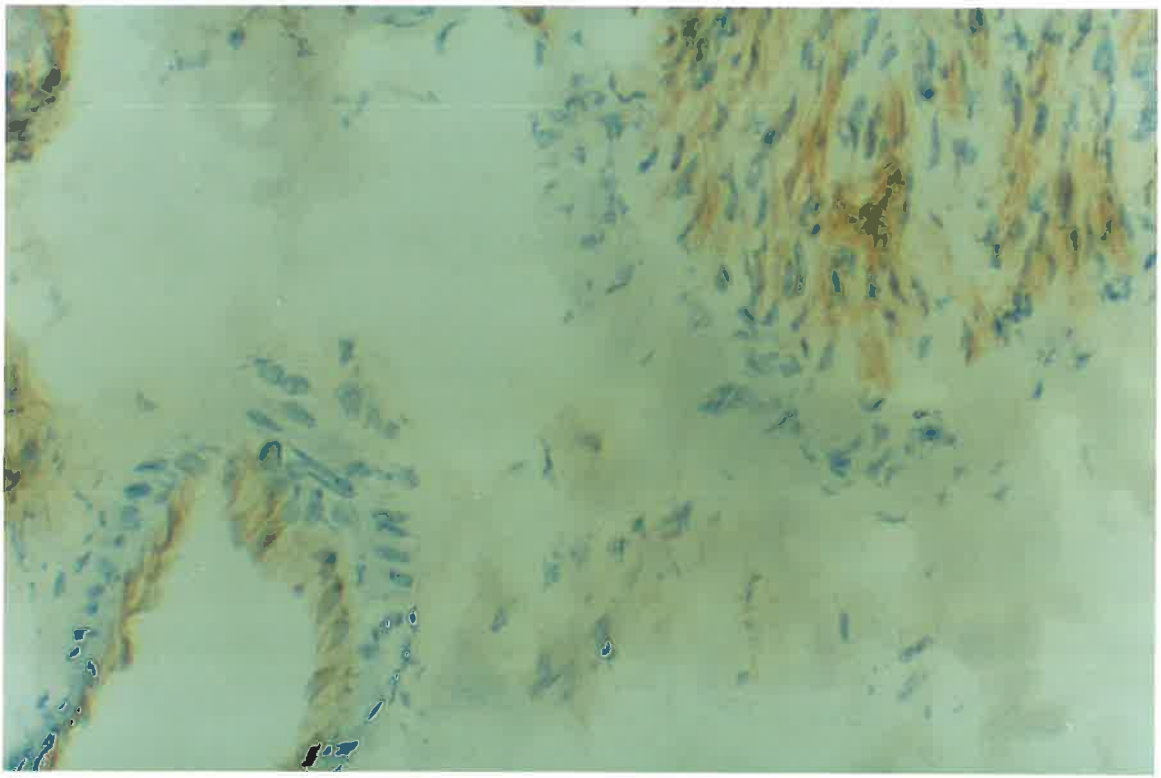


Expression of ICAM-1 on peripheral nerve (tomaculous neuropathy) (indirect immunoperoxidase, x200)



Expression of ICAM-1 in vasculitis involving skeletal muscle (indirect immunoperoxidase, x200)

Expression of ELAM-1 (1.2B6) in vasculitis involving a medium-sized artery in skeletal muscle (indirect immunoperoxidase, x200)



Expression of ICAM-1 in vasculitis involving peripheral nerve (indirect immunoperoxidase, x200)

BIBLIOGRAPHY

Abi-Hanna D, Wakefield D: HLA antigens in ocular tissues. III. Antigen presentation by gamma-interferon-treated cultured uveal cells. Human Immunology 24: 31, 1989

Ager A: Isolation and culture of high endothelial cells from rat lymph nodes. J Cell Sci 87: 133, 1987

Ager A, Mistry S: Interaction between lymphocytes and cultured high endothelial cells: an in vitro model of lymphocyte migration across high endothelial venule endothelium. Eur J Immunol 18: 1265, 1988

Akiyama SK, Yamada SS, Chen WT, Yamada KM: Analysis of fibronectin receptor function with monoclonal antibodies: roles in cell adhesion, migration, matrix assembly, and cytoskeletal organization. J Cell Biol 109: 863, 1989a

Akiyama SK, Yamada SS, Yamada KM: Analysis of the role of glycosylation of the human fibronectin receptor. J Biol Chem 264: 18011, 1989b

Albelda SM, Daise M, Levine EM, Buck CA: Identification and characterization of cell-substratum adhesion receptors on cultured human endothelial cells. J Clin Invest 83: 1992, 1989

Alexander S, Hubbard SC, Stominger JL: HLA-DR antigens of autologous melanoma and B lymphoblastoid cell lines: differences in glycosylation but not protein structure. J Immunol 133: 315, 1984

Altieri DC, Agbanyo FR, Plescia J, Ginsberg MH, Edgington TS, Plow EF: A unique recognition site mediates the interaction of fibrinogen with the leukocyte integrin Mac-1 (CD11b/CD18). J Biol Chem 265: 12119, 1990

Altmann DM, Hogg N, Trowsdale J, Wilkinson D: Cotransfection of ICAM-1 and HLA-DR reconstitutes human antigen-presenting cell function in mouse L cells. Nature 338: 512, 1989

Amiot M, Bernard A, Tran HC, Leca G, Kanellopoulos JM, Boumsell L: The human cell surface glycoprotein complex (gp 120, 200) recognized by monoclonal antibody K20 is a component binding to phytohaemagglutinin on T cells. Scand J Immunol 23: 109, 1986

Anders E, Alles JU, Delvos U, Potzsch B, Preissner KT, Muller-Berghaus G: Microvascular endothelial cells from human omental tissue: modified method for long-term cultivation and new aspects of characterisation. Microvascular Research 34: 239, 1987

Anderson DC, Miller LJ, Schmalstieg FC, Rothlein R, Springer TA: Contributions of the Mac-1 glycoprotein family to adherence-dependent granulocyte functions: structure-function assessment employing subunit-specific monoclonal antibodies. J Immunol 137: 15, 1986

Anderson DC, Springer TA: Leukocyte adhesion deficiency: an inherited defect in the Mac-1, LFA-1, and p150,95 glycoproteins. Ann Rev Med 38: 175, 1987

Andrews P, Milsom DW, Ford WL: Migration of lymphocytes across specialized vascular endothelium. V. Production of a sulphated macromolecule by high endothelial cells in lymph nodes. J Cell Sci 57: 277, 1982

Andrews P, Milsom DW, Stoddart RW: Glycoconjugates from high endothelial cells. 1. Partial characterization of a sulphated glycoconjugate from the high endothelial cells of rat lymph nodes. J Cell Sci 59: 231, 1983

Appleyard ST, Dubowitz V, Dunn MJ, Rose ML: Increased expression of HLA ABC class I antigens by muscle fibres in Duchenne muscular dystrophy, inflammatory myopathy, and other neuromuscular disorders. Lancet 1: 361, 1985

Arfors KE, Lundberg C, Lindblom L, Lundberg K, Beatty PG, Harlan JM: A monoclonal antibody to the membrane glycoprotein complex CD18 inhibits polymorphonuclear leukocyte accumulation and plasma leakage in vivo. Blood 69: 338, 1987

Argraves WS, Suzuki S, Arai H, Thompson K, Pierschbacher MD, Ruoslahti E: Amino acid sequence of the human fibronectin receptor. J Cell Biol 105: 1183, 1987

Argraves WS, Dickerson K, Burgess WH, Ruoslahti E: Fibulin, a novel protein that interacts with the fibronectin receptor β subunit cytoplasmic domain. Cell 58: 623, 1989

Arnaout MA, Spits H, Terhorst C, Pitt J, Todd RF: Deficiency of a leukocyte surface glycoprotein (LFA-1) in two patients with Mo 1 deficiency. Effects of cell activation on Mo1/ LFA-1 surface expression in normal and deficient leukocytes. J Clin Invest 74: 1291, 1984

Arnaout MA, Hakim RM, Todd RF, Dana N, Colten HR: Increased expression of an adhesion-promoting surface glycoprotein in the granulocytopenia of hemodialysis. NEJM 312: 457, 1985

Aruffo A, Stamenkovic I, Melnick M, Underhill CB, Seed B: CD44 is the principal cell surface receptor for hyaluronate. Cell 61: 1303, 1990

Asch AS, Barnwell J, Silverstein RL, Nachman RL: Isolation of the thrombospondin membrane receptor. J Clin Invest 79: 1054, 1987

Ashida ER, Johnson AR, Lipsky PE: Human endothelial cell-lymphocyte interaction. Endothelial cells function as accessory cells necessary for mitogen-induced human T lymphocyte activation in vitro. J Clin Invest 67: 1490, 1981

Astaldi GCB, Janssen MC, Lansdorp P, Willems C, Zeijlemaker WP, Oosterhof F: Human endothelial culture supernatant (HECS): A growth factor for hybridomas. J Immunol 125: 1411, 1980

Austyn JM: Lymphoid dendritic cells. Immunology 62: 161, 1987

Austyn JM, Larsen CP: Migration patterns of dendritic leucocytes. Implications for transplantation. Transplantation 49: 1, 1990

Axberg I, Ramstedt U, Patarroyo M, Beatty P, Wigzell H: Inhibition of natural killer cell cytotoxicity by a monoclonal antibody directed against adhesion-mediating protein gp90 (CD18). Scand J Immunol 26: 547, 1987

Bach FH, Bach ML, Sondel PM: Differential functions of major histocompatibility complex antigens in T-lymphocyte activation. Nature 259: 273, 1976

Bagby GC, Dinarello CA, Wallace P, Wagner C, Hefeneider S, McCall E: Interleukin 1 stimulates granulocyte macrophage colony-stimulating activity release by vascular endothelial cells. J Clin Invest 78: 1316, 1986

Bagnasco M, Pesce G, Pronzato C, Canonica GW: Functional involvement of the LFA-1/ICAM-1 adhesion system in the autologous mixed lymphocyte reaction. Cell Immunol 128: 362, 1990

Bainton DF, Miller LJ, Kishimoto TK, Springer TA: Leukocyte adhesion receptors are stored in peroxidase-negative granules of human neutrophils. J Exp Med 166: 1641, 1987

Bangham CRM, McMichael AJ: Nosing ahead in the cold war. Nature 344: 16, 1990

Bank I, Hemler M, Brenner MB, Cohen D, Levy V, Belko J, Crouse C, Chess L: A novel monoclonal antibody, 1B3.1, binds to a new epitope of the VLA-1 molecule. Cell Immunol 122: 416, 1989

Barnwell JW, Ockenhouse CF, Knowles DM: Monoclonal antibody OKM5 inhibits the in vitro binding of Plasmodium falciparum-infected erythrocytes to monocytes, endothelial, and C32 melanoma cells. J Immunol 135: 3494, 1985

Barton RW, Rothlein R, Ksiazek J, Kennedy C: The effect of anti-intercellular adhesion molecule-1 on phorbol ester-induced rabbit lung inflammation. J Immunol 143: 1278, 1989

Basson CT, Knowles WJ, Bell L, Albelda SM, Castronova V, Liotta LA, Madri JA: Spatiotemporal segregation of endothelial cell integrin and non-integrin extracellular matrix-binding proteins during adhesion events. J Cell Biol 110: 789, 1990

Baume D, Kuentz M, Pico JL, Beaujean F, Cordonnier C, Vernant JP, Hayat M, Bernard A: Failure of a CD18/anti-LFA-1 monoclonal antibody infusion to prevent graft rejection in leukaemic patients receiving T-depleted allogeneic bone marrow transplantation. Transplantation 47: 472, 1989

Beatty PG, Ledbetter JA, Martin PJ, Price TH, Hansen JA: Definition of a common leukocyte cell-surface antigen (Lp 95-150) associated with diverse cell-mediated immune functions. J Immunol 131: 2913, 1983

Beatty PG, Harlan JM, Rosen H, Hansen JA, Ochs HD, Price TH, Taylor RF, Klebanoff SJ: Absence of monoclonal-antibody-defined protein complex in boy with abnormal leucocyte function. Lancet 1: 535, 1984

Becker GJ, Hancock WW, Kraft N, Lanyon HC, Atkins RC: Monoclonal antibodies to human macrophages and leucocyte common antigens. Pathology 13: 669, 1981

Bednarczyk JL, McIntyre BW: A monoclonal antibody to VLA-4 α-chain (CDw49d) induces homotypic lymphocyte aggregation. J Immunol 144: 777, 1990

Beller DI, Springer TA, Schreiber RD: Anti-Mac-1 selectively inhibits the mouse and human type three complement receptor. J Exp Med 156: 1000, 1982

Bender JR, Pardi R, Kosek J, Engleman EG: Evidence that cytotoxic lymphocytes alter and traverse allogeneic endothelial cell monolayers. Transplantation 47: 1047, 1989

Berendt AR, Simmons DL, Tansey J, Newbold CI, Marsh K: Intercellular adhesion molecule-1 is an endothelial cell adhesion receptor for Plasmodium falciparum. Nature 341: 57, 1989

Berg EL, Goldstein LA, Jutila MA, Nakache M, Picker LJ, Streeter PR, Wu NW, Zhou D, Butcher EC: Homing receptors and vascular addressins: cell adhesion molecules that direct lymphocyte traffic. Immunol Rev 108: 5, 1989

Bevilacqua MP, Amrani D, Mosessen MW, Bianco C: Receptors for cold-insoluble globulin (plasma fibronectin) on human monocytes. J Exp Med 153: 42, 1981

Bevilacqua MP, Pober JS, Majeau GR, Cotran RS, Gimbrone MA: Interleukin 1 (IL-1) induces biosynthesis and cell surface expression of procoagulant activity in human vascular endothelial cells. J Exp Med 160: 618, 1984

Bevilacqua MP, Pober JS, Wheeler ME, Cotran RS, Gimbrone MA: Interleukin 1 acts on cultured human vascular endothelium to increase the adhesion of polymorphonuclear leukocytes, monocytes, and related leukocyte cell lines. J Clin Invest 76: 2003, 1985a

Bevilacqua MP, Pober JS, Wheeler ME, Cotran RS, Gimbrone MA: Interleukin-1 activation of vascular endothelium: effects on procoagulant activity and leucocyte adhesion. Am J Path 121: 394, 1985b

Bevilacqua MP, Pober JS, Majeau GR, Fiers W, Cotran RS, Gimbrone MA: Recombinant tumour necrosis factor induces procoagulant activity in cultured human vascular endothelium: characterisation and comparison with the actions of interleukin-1. Proc Natl Acad Sci USA 83: 4533, 1986

Bevilacqua MP, Schleef RR, Gimbrone MA, Loskutoff DJ: Regulation of the fibrinolytic system of cultured human vascular endothelium by interleukin 1. J Clin Invest 78: 587, 1986

Bevilacqua MP, Pober JS, Mendrick DL, Cotran RS, Gimbrone MA: Identification of an inducible endothelial-leukocyte adhesion molecule. Proc Natl Acad Sci USA 84: 9238, 1987

Bevilacqua MP, Stengelin S, Gimbrone MA, Seed B: Endothelial leukocyte adhesion molecule 1: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. Science 243: 1160, 1989

Bienz D, Clemetson KJ: Human platelet glycoprotein Ia. One component is only expressed on the surface of activated platelets and may be a granule constituent. J Biol Chem 264: 507, 1989

Bierer BE, Peterson A, Gorga JC, Herrman SH, Burakoff SJ: Synergistic T cell activation via the physiological ligand for CD2 and the T cell receptor. J Exp Med 168: 1145, 1988a

Bierer BE, Peterson A, Barbosa J, Seed B, Burakoff SJ: Expression of the T-cell surface molecule CD2 and an epitope-loss CD2 mutant to define the role of lymphocyte function-associated antigen 3 (LFA-3) in T-cell activation. Proc Natl Acad Sci USA 85: 1194, 1988b

Billaud M, Rousset F, Calender A, Cordier M, Aubry JP, Laisse V, Lenoir GM: Low expression of lymphocyte function-associated antigen (LFA)-1 and LFA-3 adhesion molecules is a common trait in Burkitt's lymphoma associated with and not associated with Epstein-Barr virus. Blood 75: 1827, 1990

Bishop DK, Jutila MA, Sedmak DD, Beattie MS, Orosz CG: Lymphocyte entry into inflammatory tissues in vivo. Qualitative differences of high endothelial venule-like vessels in sponge matrix allografts vs. isografts. J Immunol 142: 4219, 1989

Bishop GA, Hall BM, Suranyi MG, Tiller DJ, Horvath JS, Duggin GG: Expression of HLA antigens on renal tubular cells in culture. 1. Evidence that mixed lymphocyte culture supernatants and gamma interferon increase both class I and class II antigens. Transplantation 42: 671, 1986

Bishop GA, Waugh JA, Hall BM: Expression of HLA antigens on renal tubular cells in culture. II. Effect of increased HLA antigen expression on tubular cell stimulation of lymphocyte activation and on their vulnerability to cell-mediated lysis. Transplantation 46: 303, 1988

Bishop GA, Hall BM: Expression of leucocyte and lymphocyte adhesion molecules in the human kidney. Kidney International 36: 1078, 1989a

Bishop GA, Waugh JA, Landers DV, Krensky AM, Hall BM: Microvascular destruction in renal transplant rejection. Transplantation 48: 408, 1989b

Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC: Structure of the human class I histocompatibility antigen, HLA-A2. Nature 329: 506, 1987

Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC: The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. Nature 329: 512, 1987

Bland P: MHC class II expression by the gut epithelium. Immunology Today 9: 174, 1988

Bochner BS, Peachell PT, Brown KE, Schleimer RP: Adherence of human basophils to cultured umbilical vein endothelial cells. J Clin Invest 81: 1355, 1988

Bockenstedt LK, Goldsmith MA, Dustin M, Olive D, Springer TA, Weiss A: The CD2 ligand LFA-3 activates T cells but depends on the expression and function of the antigen receptor. J Immunol 141: 1904, 1988

Bodary SC, McLean JW: The integrin β_1 subunit associates with the vitronectin receptor α_v subunit to form a novel vitronectin receptor in a human embryonic kidney cell line. J Biol Chem 265: 5938, 1990

de Boer M, Roos D: Metabolic comparison between basophils and other leukocytes from human blood. J Immunol 136: 3447, 1986

Bogaert T, Brown N, Wilcox M: The Drosophila PS2 antigen is an invertebrate integrin that, like the fibronectin receptor, becomes localized to muscle attachments. Cell 51: 929, 1987

Bohnsack JF, Akiyama SK, Damsky CH, Knape WA, Zimmerman GA: Human neutrophil adherence to laminin in vitro. Evidence for a distinct neutrophil integrin receptor for laminin. J Exp Med 171: 1221, 1990

Bonfanti R, Furie BC, Furie B, Wagner DD: PADGEM (GMP 140) is a component of Weibel-Palade bodies of human endothelial cells. Blood 73: 1109, 1989

Bonneville M, Moreau JF, Blokland E, Pool J, Moisan JP, Goulmy E, Soulillou JP: T lymphocyte cloning from rejected human kidney allografts. Recognition repertoire of alloreactive T cell clones. J Immunol 141: 4187, 1988

Bos JD, Zonneveld I, Das PK, Krieg SR, van der Loos CM, Kapsenberg ML: The skin immune system (SIS): distribution and immunophenotype of lymphocyte subpopulations in normal human skin. J Invest Dermatol 5: 569, 1987

Bottazzo GF, Pujol-Borrell R, Hanafusa T, Feldmann M: Role of aberrant HLA-DR expression and antigen presentation in induction of endocrine autoimmunity. Lancet 2: 1115, 1983

Boucaut JC, Darribere T, Boulekbache H, Thiery JP: Prevention of gastrulation but not neuralation by antibodies to fibronectin in amphibian embryos. Nature 307: 364, 1984a

Boucaut JC, Darribere T, Paole TJ, Aoyama H, Yamada KM, Thiery JP: Biologically active synthetic peptides as probes of embryonic development: a competitive peptide inhibitor of fibronectin function inhibits gastrulation in amphibian embryos and neural crest migration in avian embryos. J Cell Biol 99: 1322, 1984b

Boukerche H, Berthier-Vergnes O, Bailly M, Dare JF, Leung LLK, McGregor JL: A monoclonal antibody (LYP18) directed against the blood platelet glycoprotein IIb/IIIa complex inhibits human melanoma growth in vivo. Blood 74: 909, 1989

Bourdon MA, Ruoslahti E: Tenascin mediates cell attachment through an RGD-dependent receptor. J Cell Biol 108: 1149, 1989

Bowen BR, Nguyen T, Lasky LA: Characterization of a human homologue of the murine peripheral lymph node homing receptor. J Cell Biol 109: 421, 1989

Bowen BR, Fennie C, Lasky LA: The MEL 14 antibody binds to the lectin domain of the murine peripheral lymph node homing receptor. J Cell Biol 110: 147, 1990

Boyd AW, Wawryk SO, Burns GF, Fecondo JV: Intercellular adhesion molecule-1 (ICAM-1) has a central role in cell-cell contact-mediated immune mechanisms. Proc Natl Acad Sci USA 85: 3095, 1988

Boyum A: Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation and of granulocytes by combining centrifugation and sedimentation at 1g. Scand J Lab Invest 21 (Suppl): 77, 1968

Brasile L, Zerbe T, Rabin B, Clarke J, Abrams A, Cerilli J: Identification of the antibody to vascular endothelial cells in patients undergoing cardiac transplantation. Transplantation 40: 672, 1985

Brasile L, Kremer JM, Clarke JL, Cerilli J: Identification of an autoantibody to vascular endothelial cell-specific antigens in patients with systemic vasculitis. Am J Med 87: 74, 1989

Breitmeyer JB: How T cells communicate. Nature 329: 760, 1987

Bretscher MS: Endocytosis and recycling of the fibronectin receptor in CHO cells. EMBO J 8: 1341, 1989

Breviario F, Bertocchi F, Dejana E, Bussolino F: IL-1-induced adhesion of polymorphonuclear leukocytes to cultured human endothelial cells. Role of platelet-activating factor. J Immunol 141: 3391, 1988

Broudy VC, Kaushansky K, Segal GM, Harlan JM, Adamson JW: Tumor necrosis factor type α stimulates human endothelial cells to produce granulocyte/macrophage colony-stimulating factor. Proc Natl Acad Sci USA 83: 7467, 1986

Broudy VC, Harlan JM, Adamson JW: Disparate effects of tumor necrosis factor-α/cachectin and tumor necrosis factor-β/lymphotoxin on haematopoietic growth factor production and neutrophil adhesion molecule expression by cultured human endothelial cells. J Immunol 138: 4298, 1987a

Broudy VC, Kaushansky K, Harlan JM, Adamson JW: Interleukin 1 stimulates human endothelial cells to produce granulocyte-macrophage colony-stimulating factor and granulocyte colony-stimulating factor. J Immunol 139: 464, 1987b

Brower DL, Jaffe SM: Requirement for integrins during Drosophila wing development. Nature 342: 285, 1989

Brown DL, Phillips DR, Damsky CH, Charo IF: Synthesis and expression of the fibroblast fibronectin receptor in human monocytes. J Clin Invest 84: 366, 1989

Brown EJ, Goodwin JL: Fibronectin receptors of phagocytes. Characterisation of the Arg-Gly-Asp binding proteins of human monocytes and polymorphonuclear leukocytes. J Exp Med 167: 777, 1988

Brown JH, Jardetzky T, Saper MA, Samraoui B, Bjorkman PJ, Wiley DC: A hypothetical model of the foreign antigen binding site of class II histocompatibility molecules. Nature 332: 845, 1988

Brown MH, Cantrell DA, Brattsand G, Crumpton MJ, Gullberg M: The CD2 antigen associates with the T-cell antigen receptor CD3 antigen complex on the surface of human T lymphocytes. Nature 339: 551, 1989

Brown PJ, Juliano RL: Selective inhibition of fibronectin-mediated cell adhesion by monoclonal antibodies to a cell-surface glycoprotein. Science 228: 1448, 1985

Brown PJ, Juliano RL: Monoclonal antibodies to distinctive epitopes on the α and β subunits of the fibronectin receptor. Exp Cell Res 177: 303, 1988

Buchanan MR, Crowley CA, Rosin RE, Gimbrone MA, Babier BM: Studies on the interaction between GP-180-deficient neutrophils and vascular endothelium. Blood 60: 160, 1982

Buck CA, Shea E, Duggan K, Horwitz AF: Integrin (the CSAT antigen): functionality requires oligomeric integrity. J Cell Biol 103: 2421, 1986

Buck CA, Horwitz AF: Cell surface receptors for extracellular matrix molecules. Ann Rev Cell Biol 3: 179, 1987

Budd RC, Cerottini JC, Horvath C, Bron C, Pedrazzini T, Howe RC, MacDonald HR: Distinction of virgin and memory T lymphocytes. Stable acquisition of the Pgp-1 glycoprotein concomitant with antigenic stimulation. J Immunol 138: 3120, 1987

Burger DR, Vetto RM: Hypothesis. Vascular endothelium as a major participant in T-lymphocyte immunity. Cell Immunol 70: 357, 1982

Burgess WH, Mehlman T, Friesel R, Johnson WV, Maciag T: Multiple forms of endothelial cell growth factor. Rapid isolation and biological and chemical characterization. J Biol Chem 260: 11389, 1985

Burn P, Kupfer A, Singer SJ: Dynamic membrane-cytoskeletal interactions: specific association of integrin and talin arises in vivo after phorbol ester treatment of peripheral blood lymphocytes. Proc Natl Acad Sci USA 85: 497, 1988

Burnette W: "Western Blotting": electrophoretic transfer of proteins from sodium dodecyl sulphate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radio-iodinated protein A. Anal Biochem 112: 195, 1981

Burns GF, Cosgrove L, Triglia T, Beall JA, Lopez AF, Werkmeister JA, Begley CG, Haddad AP, d'Apice AJF, Vadas MA, Cawley JC: The IIb-IIIa glycoprotein complex that mediates platelet aggregation is directly implicated in leucocyte adhesion. Cell 45: 269, 1986

Burns GF, Lucas CM, Krissansen GW, Werkmeister JA, Scanlon DB, Simpson RJ, Vadas MA: Synergism between membrane gangliosides and Arg-Gly-Asp-directed glycoprotein receptors in attachment to matrix proteins by melanoma cells. J Cell Biol 107: 1225, 1988

Bussolino F, Breviario F, Tetta C, Aglietta M, Mantovani A, Dejana E: Interleukin 1 stimulates platelet-activating factor production in cultured human endothelial cells. J Clin Invest 77: 2027, 1986

Bussolino F, Wang JM, Defilippi P, Turrini F, Sanavio F, Edgell CJS, Aglietta M, Arese P, Mantovani A: Granulocyte- and granulocyte-macrophage-colony stimulating factors induce human endothelial cells to migrate and proliferate. Nature 337: 471, 1989

Butcher EC, Scollay RG, Weissman IL: Organ specificity of lymphocyte migration: mediation by highly selective lymphocyte interaction with organ-specific determinants on high endothelial venules. Eur J Immunol 10: 556, 1980

Buyon JP, Slade SG, Reibman J, Abramson SB, Philips MR, Weissman G, WInchester R: Constitutive and induced phosphorylation of the α- and β-chains of the CD11/CD18 leukocyte integrin family. Relationship to adhesion-dependent functions. J Immunol 144: 191, 1990

Calucci M, Balsoni G, Lorenzet R, Pietra A, Locati D, Donati MB, Semeraro N: Cultured human endothelial cells generate tissue factor in response to endotoxin. J Clin Invest 71: 1893, 1983

Camerini D, James SP, Stamenkovic I, Seed B: Leu-8/TQ1 is the human equivalent of the MEL-14 lymph node homing receptor. Nature 342: 78, 1989

Camussi G, Bullolino F, Salvidio G, Baglioni C: Tumor necrosis factor/ cachectin stimulates peritoneal macrophages, polymorphonuclear neutrophils, and vascular endothelial cells to synthesize and release platelet-activating factor. J Exp Med 166: 1390, 1987

Cardarelli PM, Pierschbacher MD: Identification of fibronectin receptors on T lymphocytes. J Cell Biol 105: 499, 1987

Carlos TM, Schwartz BR, Kovach NL, Yee E, Rosso M, Osborn L, Chi-Rosso G, Newman B, Lobb R, Harlan JM: Vascular cell adhesion molecule-1 mediates lymphocyte adherence to cytokine-activated cultured human endothelial cells. Blood 76: 965, 1990

Carlsen E, Flatmark A, Pryde H: Cytokine-induced procoagulant activity in monocytes and endothelial cells. Further enhancement by cyclosporine. Transplantation 46: 575, 1988

Carter WG, Wayner EA: Characterization of the class III collagen receptor, a phosphorylated, transmembrane glycoprotein expressed in nucleated human cells. J Biol Chem 263: 4193, 1988

Cavender DE, Haskard DO, Joseph B, Ziff M: Interleukin 1 increases the binding of human B and T lymphocytes to endothelial cell monolayers. J Immunol 136: 203, 1986

Cavender DE, Haskard DO, Foster N, Ziff M: Superinduction of T lymphocyte-endothelial cell (EC) binding by treatment of EC with interleukin 1 and protein synthesis inhibitors. J Immunol 138: 2149, 1987a

Cavender DE, Saegusa Y, Ziff M: Stimulation of endothelial cell binding of lymphocytes by tumour necrosis factor. J Immunol 139: 1855, 1987b

Cavender DE, Haskard DO, Maliakkal D, Ziff M: Separation and characterization of human T lymphocytes with varying adhesiveness for endothelial cells. Cell Immunol 117: 111, 1988

Cavender DE, Edelbaum D, Ziff M: Endothelial cell activation induced by tumor necrosis factor and lymphotoxin. Am J Pathol 134: 551, 1989

Cerilli J, Brasile L, Galouzis T, Lempert N, Clarke J: The vascular endothelial cell antigen system. Transplantation 39: 286, 1985a

Cerilli J, Brasile L, Karmody A: Role of the vascular endothelial cell antigen system in the etiology of atherosclerosis. Ann Surg 202: 329, 1985b

Cerilli J, Brasile L, Sosa J, Kremer J, Clarke J, Leather R, Shah D: The role of autoantibody to vascular endothelial cell antigens in atherosclerosis and vascular disease. Trans Proc 19: 47, 1987

Cerilli J, Clarke J, Doolin T, Cerilli G, Brasile L: The significance of a donor-specific vessel crossmatch in renal transplantation. Transplantation 46: 359, 1988

Cerveny TJ, Fass DN, Mann KG: Synthesis of coagulation factor V by cultured aortic endothelium. Blood 63: 1467, 1984

Chan V, Chan TK: Antithrombin III in fresh and cultured human endothelial cells: a natural anticoagulant from the vascular endothelium. Thrombosis Research 15: 209, 1979

Charo IF, Yuen C, Goldstein IM: Adherence of polymorphonuclear leucocytes to endothelial monolayers: effects of temperature, divalent cations, and chemotactic factors on the strength of adherence measured with a new centrifugation assay. Blood 65: 473, 1985

Charo IF, Fitzgerald LA, Steiner B, Rall SC, Bekeart LS, Phillips DR: Platelet glycoproteins IIb and IIIa: evidence for a family of immunologically and structurally related glycoproteins in mammalian cells. Proc Natl Acad Sci USA 83: 8351, 1986

Charo IF, Bekeart LS, Phillips DR: Platelet glycoprotein IIb-IIIa-like proteins mediate endothelial cell attachment to adhesive proteins and the extracellular matrix. J Biol Chem 262: 9935, 1987

Chatila TA, Geha RS: Phosphorylation of T cell membrane proteins by activators of protein kinase C. J Immunol 140: 4308, 1988

Chen WT, Singer SJ: Immunoelectron microscopic studies of the sites of cell-substratum and cell-cell contacts in cultured fibroblasts. J Cell Biol 95: 205, 1982

Chen WT, Hasegawa E, Hasegawa T, Weinstock C, Yamada KM: Development of cell surface linkage complexes in cultured fibroblasts. J Cell Biol 100: 1103, 1985

Cheresh DA, Pierschbacher MD, Herzig MA, Mujoo K: Disialogangliosides GD2 and GD3 are involved in the attachment of human melanoma and neuroblastoma cells to extracellular matrix proteins. J Cell Biol 102: 688, 1986a

Cheresh DA, Klier FG: Disialoganglioside GD2 distributes preferentially into substrate-associated microprocesses on human melanoma cells during their attachment to fibronectin. J Cell Biol 102: 1887, 1986b

Cheresh DA, Harper JR: Arg-Gly-Asp recognition by a cell adhesion receptor requires its 130-kDa α subunit. J Biol Chem 262: 1434, 1987a

Cheresh DA, Pytela R, Pierschbacher MD, Klier FG, Ruoslahti E, Reisfeld RA: An Arg-Gly-Asp-directed receptor on the surface of human melanoma cells exists in a divalent cation-dependent functional complex with the disialoganglioside GD2. J Cell Biol 105: 1163, 1987b

Cheresh DA, Spiro RC: Biosynthetic and functional properties of an Arg-Gly-Asp-directed receptor involved in human melanoma cell attachment to vitronectin, fibrinogen, and von Willebrand factor. J Biol Chem 262: 17703, 1987c

Cheresh DA: Human endothelial cells synthesize and express an Arg-Gly-Asp-directed adhesion receptor involved in attachment to fibrinogen and von Willebrand factor. Proc Natl Acad Sci USA 84: 6471, 1987d

Cheresh DA, Smith JW, Cooper HM, Quaranta V: A novel vitronectin receptor integrin ($\alpha_v\beta_x$) is responsible for distinct adhesive properties of carcinoma cells. Cell 57: 59, 1989a

Cheresh DA, Berliner SA, Vicente V, Ruggeri ZM: Recognition of distinct adhesive sites on fibrinogen by related integrins on platelets and endothelial cells. Cell 58: 945, 1989b

Cines DB, Lyss AP, Bina M, Corkey R, Kefalides NA, Friedman HM: Fc and C3 receptors induced by herpes simplex virus on cultured human endothelial cells. J Clin Invest 69: 123, 1982

Cines DB, Lyss AP, Reeber M, Bina M, DeHoratins RJ: Presence of complement-fixing anti-endothelial cell antibodies in systemic lupus erythematosus. J Clin Invest 73: 611,1984

Clark EA, Ledbetter JA, Holly RC, Dinndorf PA, Shu G: Polypeptides on human B lymphocytes associated with cell activation. Human Immunology 16: 100, 1986

Clark RAF, Quinn JH, Winn HJ, Lanigan JM, Dellepella P, Colvin RB: Fibronectin is produced by blood vessels in response to injury. J Exp Med 156: 646,1982

Clark SC, Kamen R: The human haematopoietic colony-stimulating factors. Science 236: 1229,1987

Clayberger C, Krensky AM, McIntyre BW, Keller TD, Parham P, Brodsky F, Linn DJ, Evans EL: Identification and characterization of two novel lymphocyte function-associated antigens, L24 and L25. J Immunol 138: 1510,1987a

Clayberger C, Medeiros LJ, Link MP, Warnke RA, Wright A, Koller TD, Smith SD, Krensky AM: Absence of cell surface LFA-1 as a mechanism of escape from immunosurveillance. Lancet 2 : 533, 1987b

Cohen MC, Mecley M, Antonia SJ, Picciano PT: Adherence of tumor cells to endothelial monolayers: inhibition by lymphokines. Cell Immunol 95: 247, 1985

Coller BS, Peerschke EI, Scudder LE, Sullivan CA: Studies with a murine monoclonal antibody that abolishes ristocetin-induced binding of von Willebrand factor to platelets: additional evidence in support of GPIb as a platelet receptor for von Willebrand factor. Blood 61: 99, 1983

Coller BS: A new murine monoclonal antibody reports an activation-dependent change in the conformation and/or microenvironment of the platelet glycoprotein IIb/IIIa complex. J Clin Invest 76: 101, 1985

Coller BS: Activation affects access to the platelet receptor for adhesive glycoproteins. J Cell Biol 103: 451, 1986

Coller BS, Beer JH, Scudder LE, Steinberg MH: Collagen-platelet interactions: evidence for a direct interaction of collagen with platelet GP Ia/IIa and an indirect interaction with platelet GP IIb/IIIa mediated by adhesive proteins. Blood 74: 182, 1989

Collins T, Korman AJ, Wake CT, Boss JM, Kappes BJ, Fiers W, Ault KA, Gimbrone MA, Strominger JL, Pober JS: Immune interferon activates multiple class II major-histocompatibility complex genes and the associated invariant chain gene in human endothelial cells and dermal fibroblasts. Proc Natl Acad Sci USA 81: 4917, 1984

Colvin RB: Cellular and molecular mechanisms of allograft rejection. Ann Rev Med 41: 361, 1990

Conforti G, Zanetti A, Colella S, Abbadini M, Marchisio PC, Pytela R, Giancotti F, Tarone G, Languino LR, Dejana E: Interaction of fibronectin with cultured human endothelial cells: characterization of the specific receptor. Blood 73: 1576, 1989

Corbi AL, Miller LJ, O'Connor K, Larson RS, Springer TA: cDNA cloning and complete primary structure of the α subunit of a leukocyte adhesion glycoprotein, p150,95. EMBO J 6: 4023, 1987

Corbi AL, Larson RS, Kishimoto TK, Springer TA, Morton CC: Chromosomal location of the genes encoding the leukocyte adhesion receptors LFA-1, Mac-1, and p150,95. Identification of a gene cluster involved in cell adhesion. J Exp Med 167: 1597, 1988a

Corbi AL, Kishimoto TK, Miller LJ, Springer TA: The human leukocyte adhesion glycoprotein Mac-1 (complement receptor type 3, CD11b) α subunit. Cloning, primary structure, and relation to the integrins, von Willebrand factor and factor B. J Biol Chem 263: 12403, 1988b

Cordon-Cardo C, Bander NH, Fradet Y, Finstad CL. Whitmore WF, Lloyd KO, Oettgen HF, Melamed MR, Old LJ: Immunoanatomic dissection of the human urinary tract by monoclonal antibodies. J Histochem Cytochem 32: 1035, 1984

Cosimi AB, Conti D, Delmonico FL, Preffer FJ, Wee SL, Rothlein R, Faanes R, Colvin RB: In vivo effects of monoclonal antibody to ICAM-1 (CD54) in nonhuman primates with renal allografts. J Immunol 144: 4604, 1990

Cotran RS, Gimbone MA, Bevilacqua MP, Mendrick DL, Pober JS: Induction and detection of a human endothelial activation antigen in vivo. J Exp Med 164: 661, 1986

Cotran RS, Pober JS, Gimbone MA, Springer TA, Wiebke EA, Gaspari AA, Rosenberg SA, Lotze MT: Endothelial activation during interleukin 2 immunotherapy. A possible mechanism for the vascular leak syndrome. J Immunol 139: 1883, 1987

Courtoy PJ, Timpl R, Farquhar MG: Comparative distribution of laminin, type IV collagen, and fibronectin in the rat glomerulus. J Histochem Cytochem 30: 874, 1982

Couturier C, Haaffner-Cavaillon N, Weiss L, Fischer E, Kazatchkine MD: Induction of cell-associated interleukin I through stimulation of the adhesion -promoting proteins LFA-1 (CD11a/CD18) and CR3 (CD11b/CD18) of human monocytes. Eur J Immunol 20: 999, 1990

Cramer EB, Pologe L, Pawlowski NA, Cohn ZA, Scott WA: Leukotriene C promotes prostacyclin synthesis by human endothelial cells. Proc Natl Acad Sci USA 80: 4109, 1983

Crowley CA, Curnette JT, Rosin RE, Andre-Schwartz J, Gallin JI, Klempner M, Snyderman R, Southwick FS, Stassel TP, Babior BM: An inherited abnormality of neutrophil adhesion. Its genetic transmission and its association with a missing protein. NEJM 302: 1163, 1980

Czernielewski JM, Bagot M: Class II MHC antigen expression by human keratinocytes results from lympho-epidermal interactions and γ-interferon production. Clin Exp Immunol 66: 295, 1986

Dahl SC, Grabel LB: Integrin phosphorylation is modulated during the differentiation of F-9 teratocarcinoma stem cells. J Cell Biol 108: 183, 1989

Dahms NM, Hart GW: Influence of quaternary structure on glycosylation. Differential subunit association affects the site-specific glycosylation of the common β-subunit from Mac-1 and LFA-1. J Biol Chem 261: 13186, 1986

Damsky CH, Knudsen KA, Bradley D, Buck CA, Horwitz AF: Distribution of the cell substratum attachment (CSAT) antigen on myogenic and fibroblastic cells in culture. J Cell Biol 100: 1528, 1985

Dana N, Todd RF, Pitt J, Springer TA, Arnaout MA: Deficiency of a surface membrane protein (Mo1) in man. J Clin Invest 73: 153, 1984

Dana N, Styrt B, Griffin JD, Todd RF, Klempner MS, Arnaout MA: Two functional domains in the phagocyte membrane glycoprotein Mo1 identified with monoclonal antibodies. J Immunol 137: 3259, 1986

Dang LH, Michalek MT, Takei F, Benaceraff B, Rock KL: Role of ICAM-1 in antigen presentation demonstrated by ICAM-1 defective mutants. J Immunol 144: 4082, 1990

Dang NH, Torimoto Y, Schlossman SF, Morimoto C: Human CD4 helper T cell activation: functional involvement of two distinct collagen receptors, 1F7 and VLA integrin family. J Exp Med 172: 649, 1990

Daniel TO, Gibbs VC, Milfay DF, Williams LT: Agents that increase cAMP accumulation block endothelial c-sis induction by thrombin and transforming growth factor-β. J Biol Chem 262: 11893, 1987

Danilovs J, Ayoub G, Terasaki P: B-lymphocyte isolation by thrombin-nylon wool. IN: Histocompatibility Testing 1980. Ed: Terasaki P (UCLA Tissue Typing Laboratory, Los Angeles): 287, 1980

Das M, Soffer RL: Pulmonary angiotensin-converting enzyme. Structural and catalytic properties. J Biol Chem 250: 6762, 1975

Davies J, Warwick J, Totty N, Philp R, Helfrich M, Horton M: The osteoclast functional antigen, implicated in the regulation of bone resorption, is biochemically related to the vitronectin receptor. J Cell Biol 109: 1817, 1989

Davignon D, Martz E, Reynolds T, Kurzinger K, Springer TA: Monoclonal antibody to a novel lymphocyte function-associated antigen (LFA-1): mechanism of blockade of T lymphocyte-mediated killing and effects on other T and B lymphocyte functions. J Immunol 127: 590, 1981a

Davignon D, Martz E, Reynolds T, Kurzinger K, Springer TA: Lymphocyte function-associated antigen 1 (LFA-1): a surface antigen distinct from Lyt-2,3 that participates in T lymphocyte-mediated killing. Proc Natl Acad Sci USA 78: 4535, 1981b

Davis LS, Oppenheimer-Marks N, Bednarczyk JL, McIntyre BW, Lipsky PE: Fibronectin promotes proliferation of naive and memory T cells by signalling through both the VLA-4 and VLA-5 integrin molecules. J Immunol 145: 785, 1990

Davis MM, Bjorkman PJ: T-cell antigen receptor genes and T-cell recognition. Nature 334: 395, 1988

Davison PM, Bensch K, Karasek MA: Isolation and growth of endothelial cells from the microvessels of the newborn human foreskin in cell culture. J Invest Dermatol 75: 316, 1980

Dedhar S, Ruoslahti E, Pierschbacher MD: A cell surface receptor complex for collagen type I recognizes the Arg-Gly-Asp sequence. J Cell Biol 104: 585, 1987a

Dedhar S, Argraves WS, Suzuki S, Ruoslahti E, Pierschbacher MD: Human osteosarcoma cells resistant to detachment by an Arg-Gly-Asp-containing peptide overproduce the fibronectin receptor. J Cell Biol 105: 1175, 1987b

Dedhar S, Haqq C, Gray V: Specific overproduction of very late antigen 1 integrin in two human neuroblastoma cell lines selected for resistance to detachment by an Arg-Gly-Asp-containing synthetic peptide. J Biol Chem 264: 4832, 1989

DeFranco AL: Cell-cell interactions in the antibody response. Nature 334: 199, 1988

Dejana E, Colella S, Languino LR, Balconi G, Corbascio GC, Marchisio PC: Fibrinogen induces adhesion, spreading, and microfilament organization of human endothelial cells in vitro. J Cell Biol 104: 1403, 1987

Dejana E, Languino LR, Colella S, Corbascio GC, Plow E, Ginsberg MH, Marchisio PC: The localisation of a platelet Gp IIb-IIIa-related protein in endothelial cell adhesion structures. Blood 71: 566, 1988a

Dejana E, Colella S, Conforti G, Abbadini M, Gaboli M, Marchisio PC: Fibronectin and vitronectin regulate the organization of their respective Arg-Gly-Asp-adhesion receptors in cultured human endothelial cells. J Cell Biol 107: 1215, 1988b

Dejana E, Bertucchi F, Bortolami MC, Regonesi A, Tonta A, Brevario F, Giavazzi R: Interleukin 1 promotes tumour cell adhesion to cultured human endothelial cells. J Clin Invest 82: 1466, 1988c

Dejana E, Lampugnani MG, Giorgi M, Gaboli M, Fererici AB, Ruggeri ZM, Marchisio PC: Von Willebrand factor promotes endothelial cell adhesion via an Arg-Gly-Asp-dependent mechanism. J Cell Biol 109: 367, 1989

Dejana E, Lampugnani MG, Giorgi M, Gaboli M, Marchisio PC: Fibrinogen induces endothelial cell adhesion and spreading via the release of endogenous matrix proteins and the recruitment of more than one integrin receptor. Blood 75: 1509, 1990

Denning SM, Le PT, Singer KH, Haynes BF: Antibodies against the CD44 p80, lymphocyte homing receptor molecule augment human peripheral blood T cell activation. J Immunol 144: 7, 1990

Dennis MS, Henzel WJ, Ditti RM, Lipari MT, Napier MA, Deisher TA, Bunting S, Lazarus RA: Platelet glycoprotein IIb-IIIa protein antagonist from snake venoms: evidence for a family of platelet-aggregation inhibitors. Proc Natl Acad Sci USA 89: 2471, 1989

DeSimone DW, Hynes RO: Xenopus laevis integrins. Structural conservation and evolutionary divergance of integrin β subunits. J Biol Chem 263: 5333, 1988

Detmers PA, Wright SD, Olsen E, Kimball B, Cohn ZA: Aggregation of complement receptors on human neutrophils in the absence of ligand. J Cell Biol 105: 1137, 1987

DiCorleto PE, Bowen-Pope DF: Cultured endothelial cells produce a platelet-derived growth factor-like protein. Proc Natl Acad Sci USA 80: 1919, 1983

Diener AM, Beatty PG, Ochs HD, Harlan JM: The role of neutrophil membrane glycoprotein 150 (GP-150) in neutrophil-mediated endothelial cell injury in vitro. J Immunol 135: 537, 1985

van Dijken PJ, Ghayur T, Mauch P, Down J, Burakoff SJ, Ferrara JLM: Evidence that anti-LFA-1 in vivo improves engraftment and survival after allogeneic bone marrow transplantation. Transplantation 49: 882, 1990

Dobrina A, Schwartz BR, Carlos TM, Ochs HD, Beatty PG, Harlan JM: CD11/CD18-independent neutrophil adherence to inducible endothelial-leucocyte adhesion molecules (E-LAM) in vitro. Immunol 67: 502, 1989

Dougherty GJ, Murdock S, Hogg N: The function of human intercellular adhesion molecule-1 (ICAM-1) in the generation of an immune response. Eur J Immunol 18: 35, 1988

D'Souza SE, Ginsberg MH, Burke TA, Lam SCT, Plow EF: Localization of an Arg-Gly-Asp recognition site within an integrin adhesion receptor. Science 242: 91, 1988

D'Souza SE, Ginsberg MH, Burke TA, Plow EF: The ligand binding site of the platelet integrin receptor GPIIb-IIIa is proximal to the second calcium binding domain of its α subunit. J Biol Chem 265: 3440, 1990

D'Souza SE, Ginsberg MH, Matsueda GR, Plow EF: A discrete sequence within the platelet integrin, GPIIb-IIIa, involved in ligand recognition. Nature (in press)

Duijvestijn AM, Schreiber AB, Butcher EC: Interferon-γ regulates an antigen specific for endothelial cells involved in lymphocyte traffic. Proc Natl Acad Sci USA 83: 9114, 1986

Duijvestijn AM, Kerkhove M, Bargatze RF, Butcher EC: Lymphoid tissue- and inflammation-specific endothelial cell differentiation defined by monoclonal antibodies. J Immunol 138: 713, 1987

Duijvestijn AM, Horst E, Pals ST, Rouse BN, Steere AC, Picker LJ, Meijer CJLM, Butcher EC: High endothelial differentiation in human lymphoid and inflammatory tissues defined by monoclonal antibody HECA-452. Am J Pathol 130: 147, 1988

Duijvestijn AM, Hamann A: Mechanisms and regulation of lymphocyte migration. Immunology Today 10: 23, 1989

Dumonde DC, Pulley MS, Paradinas FJ, Southcott BM, O'Connell D, Robinson MRG, den Hollander F, Schuurs AH: Histological features of skin reactions to human lymphoid cell line lymphokine in patients with advanced cancer. J Pathol 138: 289, 1982

Dunn CJ, Fleming WE: Increased adhesion of polymorphonuclear leukocytes to vascular endothelium by specific interaction of endogenous (interleukin-1) and exogenous (lipopolysaccharide) substances with endothelial cells "in vitro". Eur J Rheum Inflamm 7: 80, 1984

Dustin ML, Rothlein R, Bhan AK, Dinarello CA, Springer TA: Induction by IL-1 and interferon-γ: tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). J Immunol 137: 245, 1986

Dustin ML, Sanders ME, Shaw S, Springer TA: Purified lymphocyte-associated antigen 3 binds to CD2 and mediates T lymphocyte adhesion. J Exp Med 165: 677, 1987a

Dustin ML, Selvaraj P, Mattaliano RJ, Springer TA: Anchoring mechanisms for LFA-3 cell adhesion glycoprotein at membrane surface. Nature 329: 846, 1987b

Dustin ML, Staunton DE, Springer TA: Supergene families meet in the immune system. Immunology Today 9: 213, 1988a

Dustin ML, Singer KH, Tuck DT, Springer TA: Adhesion of T lymphoblasts to epidermal keratinocytes is regulated by interferon γ and is mediated by intercellular adhesion molecule 1 (ICAM-1). J Exp Med 167: 1323, 1988b

Dustin ML, Springer TA: Lymphocyte function-associated antigen-1 (LFA-1) interaction with intercellular adhesion molecule-1 (ICAM-1) is one of at least three mechanisms for lymphocyte adhesion to cultured endothelial cells. J Cell Biol 107: 321, 1988c

Dustin ML, Springer TA: T-cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. Nature 341: 619, 1989

Dvorak AM, Mihm MC, Dvorak HF: Morphology of delayed-type hypersensitivity reactions in man. II. Ultrastructural alterations affecting the microvasculature and the tissue mast cells. Lab Invest 34: 179, 1976

Dvorak HF, Mihm MC, Dvorak AM, Barnes BA, Manseau EJ, Galli SJ: Rejection of first-set skin allografts in man. The microvasculature is the critical target of the immune response. J Exp Med 150: 322, 1979

Dvorak HF, Galli SJ, Dvorak AM: Cellular and vascular manifestations of cell-mediated immunity. Human Pathology 17: 122, 1986

Edgell CJS, McDonald CC, Graham JB: Permanent cell line expressing human factor VIII-related antigen established by hybridization. Proc Natl Acad Sci USA 80: 3734, 1983

Elices MJ, Hemler ME: The human integrin VLA-2 is a collagen receptor on some cells and a collagen/laminin receptor on others. Proc Natl Acad Sci USA 86: 9906, 1989

Elices MJ, Osborn L, Takada Y, Crouse C, Luhowskyj S, Hemler ME, Lobb RR: VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. Cell 60: 577, 1990

Emeis JJ, Edgell CJS: Fibrinolytic properties of a human endothelial hybrid cell line (Ea. hy 926). Blood 71: 1669, 1988

Emery P, Gentry KC, Mackay IR, Muirden KD, Rowley M: Deficiency of the suppressor inducer subset of T lymphocytes in rheumatoid arthritis. Arth Rheum 30: 849, 1987

van Epps DE, Potter J, Vachula M, Smith CW, Anderson DC: Suppression of human lymphocyte chemotaxis and transendothelial migration by anti-LFA-1 antibody. J Immunol 143: 3207, 1989

Esmon CT: The regulation of natural anticoagulant pathways. Science 235: 1348, 1987

Evans PR, Trickett LP, Smith JL, MacIver AG, Tate D, Slapak M: Variable expression of HLA-DR antigens in the human kidney. Disease Markers 2: 251, 1984

Evans PR, Trickett LP, Smith JL, MacIver AG, Tate D, Slapak M: Varying expression of major histocompatibility complex antigens on human renal endothelium and epithelium. Br J Exp Path 66: 79, 1985a

Evans PR, Trickett LP, MacIver AG, Smith JL: Heterogeneity of expression of HLA class II antigens DR, DQ, and DP on the normal human renal endothelium. Disease Markers 3: 185, 1985b

Evans PR: Use of a kidney binding assay in the selection of a donor for a highly presensitised patient. United Kingdom Transplant Service Bulletin 1985c

Falcioni R. Sacchi A, Resau J, Kennel SJ: Monoclonal antibody to human carcinoma-associated protein complex: quantitation in normal and tumor tissue. Cancer Res 48: 816, 1988

Faller DV, Kourembanas S, Ginsberg D, Hannan R, Collins T, Ewenstein BM, Pober JS, Tantravahi R: Immortalization of human endothelial cells by murine sarcoma viruses, without morphologic transformation. J Cell Physiol 134: 47, 1988

Fattorossi A, Aurbach GD, Sakaguchi K, Cama A, Marx SJ, Streeten EA, Fitzpatrick LA, Brandi ML: Anti-endothelial antibodies: detection and characterization in sera from patients with autoimmune hypoparathyroidism. Proc Natl Acad Sci USA 85: 4015, 1988

Ferraro G, Meroni PL, Tincani A, Sinico A, Barcellini W, Radice A, Gregorini G, Froldi M, Borghi MO, Balestrieri G: Anti-endothelial cell antibodies in patients with Wegener's granulomatosis and micropolyarteritis. Clin Exp Immunol 79: 47, 1990

Ferrell JE, Martin GS: Tyrosine-specific protein phosphorylation is regulated by glycoprotein IIb-IIIa in platelets. Proc Natl Acad Sci USA 86: 2234, 1989

Fibbe WE, Daha MR, Hiemstra PS, Duinkerken N, Lurvink E, Ralph P, Altrock BW, Kaushansky K, Willemze R, Fulkenburg JHF: Interleukin 1 and poly (rI).poly(rC) induce production of granulocyte CSF, macrophage CSF, and granulocyte-macrophage CSF by human endothelial cells. Exp Haematol 17: 229, 1989

Fingerman E, Hemler ME: Regulation of proteins in the VLA cell substrate adhesion family: influence of cell growth conditions on VLA-1, VLA-2, and VLA-3 expression. Exp Cell Res 177: 132, 1988

Fischer A, Descamps-Latscha B, Genota I, Scheinmetzler C, Virelizier J, Trung PH, Lisowska-Grospierre B, Perez N, Durandy A, Griscelli C: Bone-marrow transplantation for inborn error of phagocytic cells associated with defective adherence, chemotaxis, and oxidative response during opsonised particle phagocytosis. Lancet ii: 473, 1983

Fischer A, Blanche S, Veber F, Delaage M, Mawa C, Griscelli C, Le Deist F, Lopez M, Olive D, Janassy G: Prevention of graft failure by an anti-HLFA-1 monoclonal antibody in HLA-mismatched bone-marrow transplantation. Lancet 2: 1058, 1986

Fitzgerald LA, Poncz M, Steiner B, Rall SC, Bennett JS, Phillips DR: Comparison of cDNA-derived protein sequences of the human fibronectin and vitronectin receptor α-subunits and platelet glycoprotein IIb. Biochemistry 26: 8158, 1987

Flock JI, Froman G, Jonsson K, Guss B, Signas C, Nilsson B, Raucci G, Hook M, Wadstrom T, Lindberg M: Cloning and expression of the gene for a fibronectin-binding protein from Staphylococcus aureus. EMBO J 6: 2351, 1987

Folkman J, Handenschild C: Angiogenesis in vitro. Nature 288: 551, 1980

Forbes RDC, Guttman RD, Gomersall M, Hibberd J: A controlled serial ultrastructural tracer study of first-set cardiac allograft rejection in the rat. Evidence that the microvascular endothelium is the primary target of graft destruction. Am J Pathol 111: 184, 1983

Fox PL, DiCorleto PE: Regulation of production of a platelet-derived growth factor-like protein by cultured bovine aortic endothelial cells. J Cell Physiol 121: 298, 1984

Fox PL, DiCorleto PE: Fish oils inhibit endothelial cell production of platelet-derived growth factor-like protein. Science 241: 453, 1988

Frade R, Myones BL, Barel M, Krikorian L, Charriaut C, Ross GD: gp 140, a C3b-binding membrane component of lymphocytes, is the B cell C3dg/C3d receptor (CR2) and is distinct from the neutrophil C3dg receptor (CR4). Eur J Immunol 15: 1192, 1985

Fradet Y, Cordon-Cardo C, Thomson T, Daly ME, Whitmore WF, Lloyd KO, Melamed MR, Old LJ: Cell surface antigens of human bladder cancer defined by mouse monoclonal antibodies. Proc Natl Acad Sci USA 81: 224, 1984

Freed E, Gailit J, van der Geer P, Ruoslahti E, Hunter T: A novel integrin β subunit is associated with the vitronectin receptor α subunit (α_v) in a human osteosarcoma cell line and is a substrate for protein kinase C. EMBO J 8: 2955, 1989

Freedman AS, Munro JM, Rice GE, Bevilacqua MP, Morimoto C, McIntyre BW, Rhynhart K, Pober JS, Nadler LM: Adhesion of human B cells to germinal centers in vitro involves VLA-4 and INCAM-110. Science 249: 1030, 1990

Freemont AJ: A possible route for lymphocyte migration into diseased tissues. J Clin Pathol 36: 161, 1983

Freemont AJ, Ford WL: Functional and morphological changes in post-capillary venules in relation to lymphocytic infiltration into BCG-induced granulomata in rat skin. J Pathol 147: 1, 1985

Frelinger AL, Lam SCT, Plow EF, Smith MA, Loftus JC, Ginsberg MH: Occupancy of an adhesive glycoprotein receptor modulates expression of an antigenic site involved in cell adhesion. J Biol Chem 263: 12397, 1988

Frelinger AL, Cohen I, Plow EF, Smith MA, Roberts J, Lam SCT, Ginsberg MH: Selective inhibition of integrin function by antibodies specific for ligand-occupied receptor conformers. J Biol Chem 265: 6346, 1990

Fry GL, Czervionke RL, Hoak JC, Smith JB, Haycraft DL: Platelet adherence to cultured vascular cells: influence of prostacyclin (PGI_2). Blood 55: 271, 1980

Fuggle SV, Errasti P, Daar AS, Fabre JW, Ting A, Morris PJ: Localisation of major histocompatibility complex (HLA-ABC and DR) antigens in 46 kidneys: differences in HLA-DR staining of tubules among kidneys. Transplantation 35: 385, 1983

Fuggle SV, McWhinnie DL, Chapman JR, Taylor HM, Morris PJ: Sequential analysis of HLA-class II antigen expression in human renal allografts: induction of tubular class II antigens and correlation with clinical parameters. Transplantation 42: 144, 1986

Fuggle SV, McWhinnie DL, Morris PJ: Precise specificity of induced tubular HLA-class II antigens in renal allografts. Transplantation 44: 214, 1987

Gabins HJ, Springer WR, Barondes SH: Receptor for the cell binding site of Discoidin I. Cell 42: 449, 1985

Gajdusek C, Carbon S, Ross R, Nawroth P, Stern D: Activation of coagulation releases endothelial cell mitogens. J Cell Biol 103: 419, 1986

Gallatin WM, Weissman IL, Butcher EC: A cell-surface molecule involved in organ-specific homing of lymphocytes. Nature 304: 30, 1983

Gallatin WM, Wayner EA, Hoffman PA, St. John T, Butcher EC, Carter WG: Structural homology between lymphocyte receptors for high endothelium and class III extracellular matrix receptor. Proc Natl Acad Sci USA 86: 4654, 1989

Gamble JR, Harlan JM, Klebanoff SJ, Vadas MA: Stimulation of the adherence of neutrophils to umbilical vein endothelium by human recombinant tumor necrosis factor. Proc Natl Acad Sci USA 82: 8667, 1985

Gamble JR, Vadas MA: A new assay for the measurement of the attachment of neutrophils and other cell types to endothelial cells. J Immunol Methods 109: 175, 1988a

Gamble JR, Vasas MA: Endothelial adhesiveness for blood neutrophils is inhibited by transforming growth factor-β. Science 242: 97, 1988b

Gamble JR, Skinner MP, Berndt MC, Vadas MA: Prevention of activated neutrophil adhesion to endothelium by soluble adhesion protein GMP-140. Science 249: 414, 1990

Garcia-Pardo A, Wayner EA, Carter WG, Ferreira OC: Human B lymphocytes define an alternative mechanism of adhesion to fibronectin. The interaction of the $\alpha_4\beta_1$ integrin with the LHGPEILDVPST sequence of the type III connecting segment is sufficient to promote cell attachment. J Immunol 144: 3361, 1990

Gardner JM, Hynes RO: Interaction of fibronectin with its receptor on platelets. Cell 42: 439, 1985

Gartner TK, Bennett JS: The tetrapeptide analogue of the cell attachment site of fibronectin inhibits platelet aggregation and fibrinogen binding to activated platelets. J Biol Chem 260: 11891, 1985

Geboes K, Rutgeerts P, Stessens L, Vantrappen G, Desmet V: Expression of MHC class II antigens by oesophageal epithelium in herpes simplex oesophagitis. Histopathology 9: 711, 1985

Gehlsen KR, Dillner L, Engvall E, Ruoslahti E: The human laminin receptor is a member of the integrin family of cell adhesion receptors. Science 241: 1228, 1988a

Gehlsen KR, Argraves WS, Pierschbacher MD, Ruoslahti E: Inhibition of in vitro tumor cell invasion by Arg-Gly-Asp-containing synthetic peptides. J Cell Biol 106: 925, 1988b

Gehlsen KR, Dickerson K, Argraves WS, Engvall E, Ruoslahti E: Subunit stucture of a laminin-binding integrin and localization of its binding site on laminin. J Biol Chem 264: 19034, 1989

Geng JG, Bevilacqua MP, Moore KL, McIntyre TM, Prescott SM, Kim JM, Bliss GA, Zimmerman GA, McEver RP: Rapid neutrophil adhesion to activated endothelium mediated by GMP-140. Nature 343: 757, 1990

Geoffrey JS, Rosen SD: Demonstration that a lectin-like receptor (gp 90^{MEL}) directly mediates adhesion of lymphocytes to high endothelial venules of lymph nodes. J Cell Biol 109: 2463, 1989

Geppert TD, Lipsky PE: Antigen presentation by interferon-γ-treated endothelial cells and fibroblasts: differential ability to function as antigen-presenting cells despite comparable Ia expression. J Immunol 135: 3750, 1985

Geppert TD, Lipsky PE: Dissection of defective antigen presentation by interferon-γ-treated fibroblasts. J Immunol 138: 385, 1987

Giltay JC, Leeksma OC, von dem Borne AEGKr, van Mourik JA: Alloantigenic composition of the endothelial vitronectin receptor. Blood 72: 230, 1988

Giltay JC, Brinkman HJM, Modderman PW, von dem Borne AEGKr, van Mourik JA: Human vascular endothelial cells express a membrane protein complex immunochemically indistinguishable from the platelet VLA-2 (glycoprotein Ia-IIa) complex. Blood 73: 1235, 1989

Gimbrone MA, Cotran RS, Folkman J: Human vascular endothelial cells in culture. J Cell Biol 60: 673, 1974

Gimbrone MA, Fareed GC: Transformation of cultured human vascular endothelium by SV40 DNA. Cell 9: 685, 1976

Gimbrone MA, Obin MS, Brock AF, Luis EA, Hass PE, Hebert CA, Yip YK, Leung DW, Lowe DG, Kohr WJ, Darbonne WC, Bechtol KB, Baker JB: Endothelial interleukin-8: a novel inhibitor of leukocyte-endothelial interactions. Science 246: 1601, 1989

Ginsberg MH, Lightsey A, Kunicki TJ, Kaufmann A, Marguerie G, Plow EF: Divalent cation regulation of the surface orientation of platelet membrane glycoprotein IIb. Correlation with fibrinogen binding function and definition of a novel variant of Glanzmann's thrombasthenia. J Clin Invest 78: 1103, 1986

Ginsberg MH, Loftus JC, Ryckwaert JJ, Pierschbacher M, Pytela R, Ruoslahti E, Plow EF: Immunochemical and amino-terminal sequence comparison of two cytoadhesins indicates they contain similar or identical β subunits and distinct α subunits. J Biol Chem 262: 5437, 1987

Ginsberg MH, Loftus JC, Plow EF: Cytoadhesins, integrins, and platelets. Thrombosis and Haemostasis 59: 1, 1988

Glimcher LH, Kim KJ, Green I, Paul WE: Ia antigen-bearing B cell tumor lines can present protein antigen and alloantigen in a major histocompatibility complex-restricted fashion to antigen-reactive T cells. J Exp Med 155: 445, 1982

Goerdt S, Zwedlo G, Schlegel R, Hagemeier HH, Sorg C: Characterisation and expression kinetics of an endothelial cell activation antigen present in vivo only in acute inflammatory tissues. Exp Cell Biol 55: 117, 1987

Golding H, Singer A: Role of accessory cell processing and presentation of shed H-2 alloantigens in allospecific cytotoxic T lymphocyte responses. J Immunol 133: 597, 1984

Goldsmith JC, McCormick JJ: Immunologic injury to vascular endothelial cells: effects on release of prostacyclin. Blood 63: 984, 1984

Goldstein LA, Zhou DFH, Picker LJ, Minty CN, Bargatze RF, Ding JF, Butcher EC: A human lymphocyte homing receptor, the Hermes antigen, is related to cartilage proteoglycan core and link proteins. Cell 56: 1063, 1989

Gospodarowicz D, Greenburg G, Foidart JM, Savion N: The production and localization of laminin in cultured vascular and corneal endothelial cells. J Cell Physiol 107: 171, 1981

Gotch F, Rothbard J, Howland K, Townshend A, McMichael A: Cytotoxic T lymphocytes recognize a fragment of influenza virus matrix protein in association with HLA-A2. Nature 326: 881, 1987

Graber N, Gopal TV, Wilson D, Beall LD, Polte T, Newman W: T cells bind to cytokine-activated endothelial cells via a novel, inducible sialoglycoprotein and endothelial leukocyte adhesion molecule-1. J Immunol 145: 819, 1990

Graf J, Ogle RC, Robey FA, Sasaki M, Martin GR, Yamada Y, Kleinman HK: A pentapeptide from the laminin B1 chains mediates cell adhesion and binds the 67000 laminin receptor. Biochemistry 26: 6896, 1987a

Graf J, Iwamoto Y, Sasaki M, Martin GR, Kleinman HK, Robey FA, Yamada Y: Identification of an amino acid sequence in laminin mediating cell attachment, chemotaxis, and receptor binding. Cell 48: 989, 1987b

Graham RC, Shannon SL: Peroxidase arthritis II. Lymphoid cell-endothelial interactions during a developing immunologic inflammatory response. Am J Pathol 69: 7, 1972

Grant DJ, Tashiro KI, Segui-Real B, Yamada Y, Martin GR, Kleinman HK: Two different laminin domains mediate the differentiation of human endothelial cells into capillary-like structures in vitro. Cell 58: 933, 1989

Gregory CD, Murray RJ, Edwards CF, Rickinson AB: Downregulation of cell adhesion molecules LFA-3 and ICAM-1 in Epstein-Barr virus-positive Burkitt's lymphoma underlies tumour cell escape from virus-specific T cell surveillance. J Exp Med 167: 1811, 1988

Greshem HD, Goodwin JL, Allen PM, Anderson DC, Brown EJ: A novel member of the integrin receptor family mediates Arg-Gly-Asp-stimulated neutrophil phagocytosis. J Cell Biol 108: 1935, 1989

Greve JM, Gottlieb DI: Monoclonal antibodies which alter the morphology of cultured chicken myogenic cells. J Cell Biochem 18: 221, 1982

Greve JM, Davis G, Meyer AM, Forte CP, Yost SC, Marlor CN, Kamarck ME, McClelland A: The major human rhinovirus receptor is ICAM-1. Cell 56: 839, 1989

Griffin JD, Spertini O, Ernst TJ, Belvin MP, Levine HB, Kanakura Y, Tedder TF: Granulocyte-macrophage colony-stimulating factor and other cytokines regulate surface expression of the leukocyte adhesion molecule-1 on human neutrophils, monocytes, and their precursors. J Immunol 145: 576, 1990

Griffiths CEM, Voorhees JJ, Nickoloff BJ: Characterization of intercellular adhesion molecule-1 and HLA-DR expression in normal and inflamed skin: modulated by recombinant gamma interferon and tumor necrosis factor. J Am Acad Dermatol 20: 617, 1989

Groenewegen G, Buurman WA, Jeunhomme GMAA, van der Linden CJ, Vegt PA, Kootstra G: In vitro stimulation of lymphocytes by vascular endothelial cells. A study with canine arterial and venous endothelial cells. Transplantation 37: 206, 1984a

Groenewegen G, Buurman WA: Vascular endothelial cells present alloantigens to unprimed lymphocytes. Scand J Immunol 19: 269, 1984b

Groenewegen G, Buurman WA, Jeunhomme GMAA, van der Linden CJ: Effect of cyclosporine on MHC class II antigen expression on arterial and venous endothelium in vitro. Transplantation 40: 21, 1985

Gross JL, Moscatelli D, Jaffe EA, Rifkin DB: Plasminogen activator and collagenase production by cultured capillary endothelial cells. J Cell Biol 95: 974, 1982

Groux H, Huet S, Valentin H, Pham D, Bernard A: Suppressor effects and cyclic AMP accumulation by the CD29 molecule of $CD4^+$ lymphocytes. Nature 339: 152, 1989

Guan JL, Hynes RO: Lymphoid cells recognize an alternatively spliced segment of fibronectin via the integrin receptor $\alpha_4\beta_1$. Cell 60: 53, 1990

Guinan EC, Smith BR, Doukas JT, Miller RA, Pober JS: Vascular endothelial cells enhance T cell responses by markedly augmenting IL-2 concentrations. Cell Immunol 118: 166, 1989

Gullberg D, Terracio L, Rubin K: Membrane glycoproteins involved in hepatocyte adhesion to collagen type I. Exp Cell Res 175: 388, 1988

Hafler DA, Hemler ME, Christenson L, Williams JM, Shapiro HM, Strom TB, Strominger JL, Weiner HL: Investigation of in vivo activated T cells in multiple sclerosis and inflammatory central nervous system diseases. Clin Immunol Immunopathol 37: 163, 1985

Hale LP, Martin ME, McCollum DE, Nunley JA, Springer TA, Singer KA, Haynes BF: Immunohistologic analysis of the distribution of cell adhesion molecules within the inflammatory synovial microenvironment. Arth Rheum 32: 22, 1989a

Hale LP, Singer KH, Haynes BF: CD44 antibody against In (Lu)-related p80, lymphocyte-homing receptor molecule inhibits the binding of human erythrocytes to T cells. J Immunol 143: 3944, 1989b

Hajjar KA, Hajjar DP, Silverstein RL, Nachman RL: Tumor necrosis factor-mediated release of platelet-derived growth factor from cultured endothelial cells. J Exp Med 166: 235, 1987

Hajjar KA, Gavish D, Breslow JL, Nachman RL: Lipoprotein (a) modulation of endothelial cell surface fibrinolysis and its potential role in atherosclerosis. Nature 339: 303, 1988

Hall BM, Dorsch SE: Cells mediating allograft rejection. Immunol Rev 77: 31, 1984a

Hall BM, Bishop GA, Duggin GA, Horvath JS, Philips J, Tiller DJ: Increased expression of HLA-DR antigens on renal tubular cells in renal transplants: relevance to the rejection response. Lancet 2: 247, 1984b

Hall DE, Reichardt LF, Crowley E, Halley B, Moezzi H, Sonnenberg A, Damsky CH: The $\alpha_1\beta_1$ and $\alpha_6\beta_1$ integrin heterodimers mediate cell attachment to distinct sites on laminin. J Cell Biol 110: 2175, 1990

Hall HG, Farson DA, Bissell MJ: Lumen formation by epithelial cell lines in response to collagen overlay: a morphogenetic model in culture. Proc Natl Acad Sci USA 79: 4672, 1982

Halloran PF, Autenried P, Wadgymar A: Regulation of HLA antigen expression in human kidney. Clinics in Immunology and Allergy 6: 411, 1986

Hamann A, Jablonski-Westrich D, Duijvestijn A, Butcher EC, Baisch H, Harder R, Thiele HG: Evidence for an accessory role of LFA-1 in lymphocyte-high endothelium interactions during homing. J Immunol 140: 693, 1988a

Hamann A, Jablonski-Westrich D, Scholz KU, Duijvestijn A, Butcher EC, Thiele HG: Regulation of lymphocyte homing. 1. Alterations in homing receptor expression and organ-specific high endothelial venule binding of lymphocytes upon activation. J Immunol 140: 737, 1988b

Hamburger SA, McEver RP: GMP-140 mediates adhesion of stimulated platelets to neutrophils. Blood 75: 550, 1990

Hanafusa T, Chiovato L, Doniach D, Pujol-Borrell R, Russell RCG, Bottazzo GF: Aberrant expression of HLA-DR antigen on thyrocytes in Grave's disease: relevance for autoimmunity. Lancet 2: 1111, 1983

Hancock WW, Kraft N, Atkins RC: The immunohistochemical demonstration of major histocompatibility antigens in the human kidney using monoclonal antibodies. Pathology 14: 409, 1982

Hancock WW, Zola H, Atkins RC: Antigenic heterogeneity of human mononuclear phagocytes: immunohistologic analysis using monoclonal antibodies. Blood 62: 1271, 1983

Hancock WW, Cotran RS: Induction of activation antigens on human glomerular endothelium by interleukin 1 (IL-1), interferon gamma (IFN-γ), and endotoxin (LPS). Kidney International 31: 322, 1987

Harlan JM: Leukocyte-endothelial interactions. Blood 65: 513, 1985a

Harlan JM, Killen PD, Senecal FM, Schwartz BR, Yee EK, Taylor RF, Beatty PG, Price TH, Ochs HD: The role of neutrophil membrane glycoprotein GP-150 in neutrophil adherence to endothelium in vitro. Blood 66: 167, 1985b

Harlan JM, Thompson PJ, Ross RR, Bowen-Pope DF: α-Thrombin induces release of platelet-derived growth factor-like molecule(s) by cultured human endothelial cells. J Cell Biol 103: 1129, 1986

Hart DNJ, Winearls CG, Fabre JW: Graft adaptation: studies on possible mechanisms in long-term surviving rat renal allografts. Transplantation 30: 73, 1980

Hart DNJ, Fabre JW: Major histocompatibility complex antigens in rat kidney, ureter, and bladder. Localization with monoclonal antibodies and demonstration of Ia-positive dendritic cells. Transplantation 31: 318, 1981a

Hart DNJ, Fabre JW: Endogenously produced Ia antigens within cells of convoluted tubules of rat kidney. J Immunol 126: 2109, 1981b

Hart DNJ, Fuggle SV, Williams KA, Fabre JW, Ting A, Morris PJ: Localization of HLA-ABC and DR antigens in human kidney. Transplantation 31: 428, 1981c

Hashemi S, Smith CD, Izaguirre CA: Anti-endothelial cell antibodies: detection and characterization using a cellular enzyme-linked immunosorbent assay. J Lab Clin Med 109: 434, 1987

Haskard DO, Cavender D, Ziff M: Phorbol ester-stimulated T lymphocytes show enhanced adhesion to human endothelial cell monolayers. J Immunol 137: 1429, 1986a

Haskard DO, Cavender D, Beatty P, Springer T, Ziff M: T lymphocyte adhesion to endothelial cells: mechanisms demonstrated by anti-LFA-1 monoclonal antibodies. J Immunol 137: 2901, 1986b

Haskard DO, Cavender D, Fleck RM, Southeimer R, Ziff M: Human dermal microvascular endothelial cells behave like umbilical vein endothelial cells in T-cell adhesion studies. J Invest Dermatol 88: 340, 1987

Haskard DO, Strobel S, Thornhill M, Pitzalis C, Levinsky RJ: Mechanisms of lymphocyte adhesion to endothelial cells: studies using a LFA-1-deficient cell line. Immunology 66: 111, 1989

Hattori R, Hamilton KK, McEver RP, Sims PJ: Complement proteins C5b-9 induce secretion of high molecular weight multimers of endothelial von Willebrand factor and translocation of granule membrane protein GMP-140 to the cell surface. J Biol Chem 264: 9053, 1989

Haverstick DM, Cowan JF, Yamada KM, Santoro SA: Inhibition of platelet adhesion to fibronectin, fibrinogen, and von Willebrand factor substrates by a synthetic tetrapeptide derived from the cell-binding domain of fibronectin. Blood 66: 946, 1985

Hayman EG, Pierschbacher MD, Ohgren Y, Ruoslahti E: Serum spreading factor (vitronectin) is present at the cell surface and in tissues. Proc Natl Acad Sci USA 80: 4003, 1983

Hayman EG, Pierschbacher MD, Ruoslahti E: Detachment of cells from culture substrate by soluble fibronectin peptides. J Cell Biol 100: 1948, 1985

Haynes BF, Telen MJ, Hale LP, Denning SM: CD44 - a molecule involved in leukocyte adherence and T-cell activation. Immunology Today 10: 423, 1989

Hayry P: Intragraft events in allograft destruction. Transplantation 38: 1, 1984

He Q, Beyers AD, Barclay AN, Williams AF: A role in transmembrane signalling for the cytoplasmic domain of the CD2 T lymphocyte surface antigen. Cell 54: 979, 1988

Heagy W, Waltenbaugh C, Martz E: Potent ability of anti-LFA-1 monoclonal antibody to prolong allograft survival. Transplantation 37: 520, 1984

Hedin U, Bottger BA, Forsberg E, Johansson S, Thyberg J: Diverse effects of fibronectin and laminin on phenotypic properties of cultured arterial smooth cells. J Cell Biol 107: 307, 1988

Heimark RL, Schwartz SM: Binding of coagulation factors IX and X to the endothelial cell surface. Biochem Biophys Res Commun 111: 723, 1983

Heimark RL, Twardzik DR, Schwartz SM: Inhibition of endothelial regeneration by type-beta transforming growth factor from platelets. Science 233: 1078, 1986

Heino J, Ignotz RA, Hemler ME, Crouse C, Massague J: Regulation of cell adhesion receptors by transforming growth factor-β. Concomitant regulation of integrins that share a common β_1 subunit. J Biol Chem 264: 380, 1989

Hemler ME, Ware CF, Strominger JL: Characterization of a novel differentiation antigen complex recognised by a monoclonal antibody (A-1A5): unique activation-specific molecular forms on stimulated T cells. J Immunol 131: 334, 1983

Hemler ME, Sanchez-Madrid F, Flotte TJ, Krensky AM, Burakoff SJ, Bhan AK, Springer TA, Strominger JL: Glycoproteins of 210,000 and 130,000 M.W. on activated T cells: cell distribution and antigenic relation to components on resting cells and T cell lines. J Immunol 132: 3011, 1984

Hemler ME, Jacobson JG, Brenner MB, Mann D, Strominger JL: VLA-1: a T cell surface antigen which defines a novel late stage of human T cell activation. Eur J Immunol 15: 502, 1985a

Hemler ME, Jacobson JG, Strominger JL: Biochemical characterization of VLA-1 and VLA-2. Cell surface heterodimers on activated T cells. J Biol Chem 260: 15246, 1985b

Hemler ME, Glass D, Coblyn JS, Jacobson JG: Very late activation antigens on rheumatoid synovial fluid T lymphocytes: association with stages of T cell activation. J Clin Invest 78: 696, 1986

Hemler ME, Huang C, Schwarz L: The VLA protein family. Characterization of five distinct cell surface heterodimers each with a common 130,000 molecular weight β subunit. J Biol Chem 262: 3300, 1987a

Hemler ME, Huang C, Takada Y, Schwarz L, Strominger JL, Clabby ML: Characterization of the cell surface heterodimer VLA-4 and related peptides. J Biol Chem 262: 11478, 1987b

Hemler ME, Crouse C, Takada Y, Sonnenberg A: Multiple very late antigen (VLA) heterodimers on platelets. Evidence for distinct VLA-2, VLA-5 (fibronectin receptor), and VLA-6 structures. J Biol Chem 263: 7660, 1988a

Hemler ME: Adhesive protein receptors on hematopoietic cells. Immunology Today 9: 109, 1988b

Hemler ME, Crouse C, Sonnenberg A: Association of the VLA α_6 subunit with a novel protein. A possible alternative to the common VLA β_1 subunit on certain cell lines. J Biol Chem 264: 6529, 1989

Hemler ME: VLA proteins in the integrin family: structures, functions, and their role on leukocytes. Ann Rev Immunol 8: 365, 1990

Hendriks HR, Korn C, Hebins RE, Kraal G: Interferon-gamma-increased adherence of lymphocytes to high endothelial venules. Immunol 68: 221, 1989

Henson PM, Johnston RB: Tissue injury in inflammation. Oxidants, proteinases, and cationic proteins. J Clin Invest 79: 669, 1987

Herbst TJ, McCarthy JB, Tsilibary EC, Furcht LT: Differential effects of laminin, intact type IV collagen, and specific domains of type IV collagen on endothelial cell adhesion and migration. J Cell Biol 106: 1365, 1988

Hermanowski-Vosatka A, Detmers PA, Gotze O, Silverstein SC, Wright SD: Clustering of ligand on the surface of a particle enhances adhesion to receptor-bearing cells. J Biol Chem 263: 17822, 1988

Hession C, Osborn L, Goff D, Chi-Rosso G, Vassallo C, Pasek M, Pittack C, Tizard R, Goelz S, McCarthy K, Hopple S, Lobb R: Endothelial leukocyte adhesion molecule 1: direct expression cloning and functional interactions. Proc Natl Acad Sci USA 87: 1673, 1990

Heurkens AHM, Hiemstra PS, Lafeber GHM, Daha MR, Breedveld FC: Anti-endothelial cell antibodies in patients with rheumatoid arthritis complicated by vasculitis. Clin Exp Immunol 78: 7, 1989

Hibbs ML, Wardlaw AJ, Stacker SA, Anderson DC, Lee A, Roberts TM, Springer TA: Transfection of cells from patients with leukocyte adhesion deficiency with an integrin β subunit (CD18) restores lymphocyte function-associated antigen-1 expression and function. J Clin Invest 85: 674, 1990

Hickstein DD, Hickey MJ, Ozols J, Baker DH, Back AL, Roth GH: cDNA sequence for the αM subunit of the human neutrophil adherence receptor indicates homology to integrin α subunits. Proc Natl Acad Sci USA 86: 257, 1989

Hildreth JEK, Gotch FM, Hildreth PDK, McMichael AJ: A human lymphocyte-associated antigen involved in cell-mediated lympholysis. Eur J Immunol 13: 202, 1983

Hildreth JEK, August JT: The human lymphocyte function-associated (HLFA) antigen and a related macrophage differentiation antigen (HMAC-1): functional effects of subunit-specific monoclonal antibodies. J Immunol 134: 3272, 1985

van Hinsberg VWM, Kooista T, van den Berg EA, Princen HMG, Fiers W, Emeis JJ: Tumor necrosis factor increases the production of plasminogen activator inhibitor in human endothelial cells in vitro and in vivo. Blood 72: 1467, 1988

Hirschberg H, Evensen SA, Henriksen T, Thorsby E: The human mixed lymphocyte-endothelium culture interaction. Transplantation 19: 495, 1975

Hirschberg H, Bergh OJ, Thorsby E: Antigen presenting properties of human vascular endothelial cells. J Exp Med 152: 249, 1980

Hirschberg H, Braathen LR, Thorsby E: Antigen presentation by vascular endothelial cells and epidermal Langerhans cells: the role of HLA-DR. Immunol Rev 66: 57, 1982

Hirst R, Horwitz A, Buck C, Rohrschneider L: Phosphorylation of the fibronectin receptor complex in cells transformed by oncogenes that encode tyrosine kinases. Proc Natl Acad Sci USA 83: 6470, 1986

Hogervorst F, Kuikman I, von dem Borne AEGKr, Sonnenberg A: Cloning and sequence analysis of beta-4 cDNA: an integrin subunit that contains a unique 118 kd cytoplasmic domain. EMBO J 9: 765, 1990

Hogg N, Takacs L, Palmer DG, Selvendran Y, Allen C: The p150,95 molecule is a marker of human mononuclear phagocytes: comparison with expression of class II molecules. Eur J Immunol 16: 240, 1986

Holers VM, Ruff TG, Parks DL, McDonald JA, Ballard LL, Brown EJ: Molecular cloning of a murine fibronectin receptor and its expression during inflammation. Expression of VLA-5 is increased in activated peritoneal macrophages in a manner discordant from major histocompatibility complex class II. J Exp Med 169: 1589, 1989

Holt CM, Lindsey N, Moult J, Malia RG, Greaves M, Hume A, Rowell NR, Hughes P: Antibody-dependent cellular cytotoxicity of vascular endothelium: characterization and pathogenic associations in systemic sclerosis. Clin Exp Immunol 78: 359, 1989

Holthofer H, Virtanen I, Karinierui AL, Hormia M, Linder E, Miettinen A: Ulex europaeus I lectin as a marker for vascular endothelium in human tissues. Lab Invest 47: 60, 1982

Holzmann B, McIntyre BW, Weissman IL: Identification of a murine Peyer's patch-specific lymphocyte homing receptor as an integrin molecule with an α chain homologous to human VLA-4α. Cell 56: 37, 1989a

Holzmann B, Weissman IL: Peyer's patch-specific lymphocyte homing receptors consist of a VLA-4-like α chain associated with either of two integrin β chains, one of which is novel. EMBO J 6: 1735, 1989b

Hoover RL, Briggs RT, Karnovsky MJ: The adhesive interaction between polymorphonuclear leucocytes and endothelial cells in vitro. Cell 14: 423, 1978

Hopt UT, Bockhorn H, Muller GH, Thiede K, Simmons RL: Lymphocyte-recruiting capacity of cells infiltrating rejecting sponge matrix allografts in vivo. Trans Proc 15: 367, 1983

Horley KJ, Carpenito C, Baker B, Takei F: Molecular cloning of murine intercellular adhesion molecule (ICAM-1). EMBO J 8: 2889, 1989

Horwitz AF, Duggan K, Greggs R, Decker C, Buck C: The cell substrate attachment (CSAT) antigen has properties of a receptor for laminin and fibronectin. J Cell Biol 101: 2134, 1985

Horwitz AF, Duggan K, Buck C, Beckerle MC, Burridge K: Interaction of plasma membrane fibronectin receptor with talin - a transmembrane linkage. Nature 320: 531, 1986

Hosein B, Bianco C: Monocyte receptors for fibronectin characterized by a monoclonal antibody that interferes with receptor activity. J Exp Med 162: 157, 1985

Houssiau FA, Coulie PG, Olive D, van Snick J: Synergistic activation of human T cells by interleukin 1 and interleukin 6. Eur J Immunol 18: 653, 1988

Howard FD, Ledbetter JA, Wong J, Bieber CP, Stinson EB, Herzenberg LA: A human T lymphocyte differentiation marker defined by monoclonal antibodies that block E-rosette formation. J Immunol 126: 2117, 1981

Hsi BL, Yeh CJG, Samson M, Fehlmann M: Monoclonal antibody GB36 raised against human trophoblast antigen recognizes a novel epithelial antigen. Placenta 8: 209, 1987

Huber AR, Weiss SJ: Disruption of the sub-endothelial basement membrane during neutrophil diapedesis in an in vitro construct of a blood vessel wall. J Clin Invest 83: 1122, 1989

Huet S, Groux H, Caillou B, Valentin H, Prieur AM, Bernard A: CD44 contributes to T cell activation. J Immunol 143: 798, 1989

Hughes CCW, Savage COS, Pober JS: Endothelial cells augment T cell interleukin 2 production by a contact-dependent mechanism involving CD2/LFA-3 interaction. J Exp Med 171: 1453, 1990

Humphries MJ, Olden K, Yamada KM: A synthetic peptide from fibronectin inhibits experimental metastasis of murine melanoma cells. Science 233: 467, 1986

Humphries MJ, Akiyama SK, Komoriya A, Olden K, Yamada KM: Neurite extension of chicken peripheral nervous system neurons on fibronectin: relative importance of specific adhesion sites in the central cell-binding domain and the alternatively spliced type III connecting segment. J Cell Biol 106: 1289, 1988

Hunig T, Tiefenthaler G, zum Buschenfelde KHM, Meuer SC: Alternative pathway activation of T cells by binding of CD2 to its cell-surface ligand. Nature 326: 298, 1987

Hynes RO: Integrins: a family of cell surface receptors. Cell 48: 549, 1987

Idearda RL, Carter WG, Nottenburg C, Wayner EA, Gallatin WM, St John T: Isolation and DNA sequence of a cDNA clone encoding a lymphocyte adhesion receptor for high endothelium. Proc Natl Acad Sci USA 86: 4659, 1989

Ignatius MJ, Reichardt LF: Identification of a neuronal laminin receptor: an Mr 200/120 kD integrin heterodimer that binds laminin in a divalent cation-dependent manner. Neuron 1: 713, 1988

Ignotz RA, Massague J: Cell adhesion protein receptors as targets for transforming growth factor-β action. Cell 51: 189, 1987

Iguchi T, Ziff M: Electron microscopic study of rheumatoid synovial vasculature. Intimate relationship between tall endothelium and lymphoid aggregation. J Clin Invest 77: 355, 1986

Ill CR, Engvall E, Ruoslahti E: Adhesion of platelets to laminin in the absence of activation. J Cell Biol 99: 2140, 1984

Ingber DE, Folkman J: Mechanochemical switching between growth and differentiation during fibroblast growth factor-stimulated angiogenesis in vitro: role of extracellular matrix. J Cell Biol 109: 317, 1989a

Ingber DE, Folkman J: How does extracellular matrix control capillary morphogenesis? Cell 58: 803, 1989b

Isberg RR, Leong JM: Multiple β_1 chain integrins are receptors for invasin, a protein that promotes bacterial penetration into mammalian cells. Cell 60: 861, 1990

Ise Y, Yamaguchi K, Sato K, Yamamura Y, Kitamura F, Tamatani T, Miyasaka M: Molecular mechanisms underlying lymphocyte recirculation. 1. Functional, phenotypical and morphological characterization of high endothelial cells cultured in vitro. Eur J Immunol 18: 1235, 1988

Ishikawa F, Miyazono K, Hellman U, Drexler H, Wernstedt C, Hagiwara K, Usuki K, Takaku F, Risau W, Heldin CH: Identification of angiogenic activity and the cloning and expression of platelet-derived endothelial cell growth factor. Nature 338: 557, 1989

Ismail G, Morganroth ML, Todd RF, Boxer LA: Prevention of pulmonary injury in isolated perfused rat lungs by activated human neutrophils preincubated with anti-Mo1 monoclonal antibody. Blood 69: 1167, 1987

Issekutz TB, Stoltz JM, van der Meide P: Lymphocyte recruitment in delayed-type hypersensitivity. The role of IFN-gamma. J Immunol 140: 2989, 1988

Issekutz TB: Effects of six different cytokines on lymphocyte adherence to microvascular endothelium and in vivo lymphocyte migration in the rat. J Immunol 144: 2140, 1990

Iwamoto Y, Robey FA, Graf J, Sasaki M, Kleinman HK, Yamada Y, Martin GR: YIGSR, a synthetic laminin pentapeptide, inhibits experimental metastasis formation. Science 238: 1132, 1987

Jaffe EA, Nachman RL, Becker CG, Minick CR: Culture of human endothelial cells derived from umbilical veins: identification by morphologic and immunologic criteria. J Clin Inv 52: 2745, 1973a

Jaffe EA, Hoyer LW, Nachman RL: Synthesis of antihaemophilic factor antigen by cultured human endothelial cells. J Clin Inv 52: 2757, 1973b

Jaffe EA, Hoyer LW, Nachman RL: Synthesis of von Willebrand factor by cultured human endothelial cells. Proc Natl Acad Sci USA 71: 1906, 1974

Jaffe EA, Mosher DF: Synthesis of fibronectin by cultured human endothelial cells. J Exp Med 147: 1779, 1978

Jaffe EA, Weksler BB: Recovery of endothelial cell prostacyclin production after inhibition by low doses of aspirin. J Clin Invest 63: 532, 1979

Jaffe EA: Cell biology of endothelial cells. Human Pathology 18: 234, 1987

Jalkanen ST, Butcher EC: In vitro analysis of the homing properties of human lymphocytes: developmental regulation of functional receptors for high endothelial venules. Blood 66: 577, 1985

Jalkanen ST, Bargatze RF, Herron LR, Butcher EC: A lymphoid cell surface glycoprotein involved in endothelial cell recognition and lymphocyte homing in man. Eur J Immunol 16: 1195, 1986a

Jalkanen ST, Steere AC, Fox RI, Butcher EC: A distinct endothelial cell recognition system that controls lymphocyte traffic into inflamed synovium. Science 233: 556, 1986b

Jalkanen ST, Bargatze RF, de los Toyos J, Butcher EC: Lymphocyte recognition of high endothelium: antibodies to distinct epitopes of an 85-95-kD glycoprotein antigen differentially inhibit lymphocyte binding to lymph node, mucosal, or synovial endothelial cells. J Cell Biol 105: 983, 1987

Jalkanen ST, Jalkanen M, Bargatze RF, Tammi M, Butcher EC: Biochemical properties of glycoproteins involved in lymphocyte recognition of high endothelial venules in man. J Immunol 141: 1615, 1988

James SP, Fiocchi C, Graeff AS, Strober W: Phenotypic analysis of lamina propria lymphocytes. Predominance of helper-inducer and cytolytic T-cell phenotypes and deficiency of suppressor-inducer phenotypes in Crohn's disease and control patients. Gastroenterology 91: 1483, 1986

Jaspers M, de Strooper B, Spaepen M, van Leuven F, David G, van den Berghe H, Cassiman JJ: Post-translational modification of the β-subunit of the human fibronectin receptor. FEBS Letters 231: 402, 1988

Jephthah-Ochola J, Urmson J, Farkus S, Halloran PF: Regulation of MHC expression in vivo. Bacterial lipopolysaccharide induces class I and class II MHC products in mouse tissues by a T-cell independent, cyclosporine-sensitive mechanism. J Immunol 141: 792, 1988

Jirik FR, Podor TJ, HiranoT, Kishimoto T, Loskutoff DJ, Carson DA, Lotz M: Bacterial lipopolysaccharide and inflammatory mediators augment IL-6 secretion by human endothelial cells. J Immunol 142: 144,1989

Johnson JP, Stade BG, Hupka U, Holzmann B, Riethmuller G: The melanoma progression-associated antigen P3.58 is identical to the intercellular adhesion molecule, ICAM-1. Immunobiology 178: 275, 1988

Johnson JP, Stade BG, Holzmann B, Schwable W, Riethmuller G: De novo expression of intercellular-adhesion molecule 1 in melanoma correlates with increased risk of metastasis. Proc Natl Acad Sci USA 86: 641, 1989

Johnston GI, Kurosky A, McEver RP: Structural and biosynthetic studies of the granule membrane protein, GMP-140, from human platelets and endothelial cells. J Biol Chem 264: 1816, 1989a

Johnston GI, Cook RG, McEver RP: Cloning of GMP-140, a granule membrane protein of platelets and endothelium: sequence similarity to proteins involved in cell adhesion and inflammation. Cell 56: 1033, 1989b

Johnston SC, Dustin ML, Hibbs ML, Springer TA: On the species specificity of the interaction of LFA-1 with intercellular adhesion molecules. J Immunol 145: 1181, 1990

Jordan SC, Yap HK, Sakai RS, Alfonso P, Fitchman M: Hyperacute allograft rejection mediated by anti-vascular endothelial cell antibodies with a negative monocyte crossmatch. Transplantation 46: 585, 1988

Jurgensen CH, Huber BE, Zimmerman TP, Wolberg G: 3-Deazaadenosine inhibits leukocyte adhesion and ICAM-1 biosynthesis in tumor necrosis factor-stimulated human endothelial cells. J Immunol 144: 653, 1990

Jutila MA, Berg EL, Kishimoto TK, Picker LJ, Bargatze RF, Bishop DK, Orosz CG, Wu NW, Butcher EC: Inflammation-induced endothelial cell adhesion to lymphocytes, neutrophils, and monocytes. Role of homing receptors and other adhesion molecules. Transplantation 48: 727, 1989a

Jutila MA, Rott L, Berg EL, Butcher EC: Function and regulation of the neutrophil MEL-14 antigen in vivo: comparison with LFA-1 and Mac-1. J Immunol 143: 3318, 1989b

Kabelitz D: Do CD2 and CD3-TCR T-cell activation pathways function independently? Immunology Today 2: 44, 1990

Kaiser L, Sparks HV: Endothelial cells: not just a cellophane wrapper. Arch Intern Med 147: 569, 1987

Kajiji SM, Davceva B, Quaranta V: Six monoclonal antibodies to human pancreatic cancer antigens. Cancer Research 47: 1367, 1987

Kajiji S, Tamura RN, Quaranta V: A novel integrin ($\alpha_E\beta_4$) from human epithelial cells suggests a fourth family of integrin adhesion receptors. EMBO J 3: 673, 1989

Kalish RS, Morimoto C, Schlossman SF: Generation of CD8 (T) cytotoxic cells has a preferential requirement for $CD4^+2H4^-$ inducer cells. Cell Immunol 111: 379, 1988

Kamoun M, Martin PJ, Hansen JA, Brown MA, Siadak AW, Nowinski RC: Identification of a human T lymphocyte surface protein associated with the E-rosette receptor. J Exp Med 153: 207, 1981

Kansas GS, Wood GS, Dailey MO: A family of cell-surface glycoproteins defined by a putative anti-endothelial cell receptor antibody in man. J Immunol 142: 3050, 1989

Kantor RRS, Matter MJ, Lloyd KO, Old LJ, Albino AP: Biochemical analysis of two cell surface glycoprotein complexes, very common antigen 1 and very common antigen 2. Relationship to very late activation T cell antigens. J Biol Chem 262: 15158, 1987

Kao K, Pizzo SV, McKee PA: Demonstration and characterization of specific binding sites for factor VIII/von Willebrand factor on human platelets. J Clin Invest 63: 656, 1979

Karpatkin S, Pearlstein E, Ambrogio C, Coller BS: Role of adhesive proteins in platelet tumor interaction in vitro and metastasis formation in vivo. J Clin Invest 81: 1012, 1988

Kaufmann R, Frosch D, Wertphal C, Weber L, Klein CE: Integrin VLA-3: ultrastructural localization at cell-cell contact sites of human cell cultures. J Cell Biol 109: 1807, 1989

Kawakami K, Yamamoto Y, Kakimoto K, Onoue K: Requirement for delivery of signals by physical interaction and soluble factors from accessory cells in the induction of receptor-mediated T cell proliferation. Effectiveness of IFN-γ modulation of accessory cells for physical interaction with T cells. J Immunol 142: 1818, 1989

Kehrel B, Balleisen L, Kokott R, Mesters R, Stenzinger W, Clemetson KJ, van de Loo J: Deficiency of intact thrombospondin and membrane glycoprotein Ia in platelets with defective collagen-induced aggregation and spontaneous loss of disorder. Blood 71: 1074, 1988

Keizer GD, Figdor CG, de Vries JE: Sensitive and quantitative determination of monocyte adherence. J Immunol Methods 95: 141, 1986

Keizer GD, Borst J, Visser W, Schwarting R, de Vries JE, Figdor CG: Membrane glycoprotein p150,95 of human cytotoxic T cell clones is involved in conjugate formation with target cells. J Immunol 138: 3130, 1987

Keizer GD, Visser W, Vliem M, Figdor CG: A monoclonal antibody (NKI-L16) directed against a unique epitope on the α-chain of human leukocyte function-associated antigen-1 induces homotypic cell-cell interactions. J Immunol 140: 1393, 1988

Kennel SJ, Foote LJ, Lankford PK: Analysis of surface proteins of mouse lung carcinomas using monoclonal antibodies. Cancer Res 41: 3465, 1981

Kennel SJ, Foote LJ, Falcioni R, Sonnenberg A, Stringer CD, Crouse C, Hemler ME: Analysis of the tumor-associated antigen TSP-180. Identity with α_6-β_4 in the integrin superfamily. J Biol Chem 264: 15515, 1989

Kerjaschki D, Ojha PP, Susani M, Horvat R, Binder R, Hovorka A, Hillemanns P, Pytela R: A β_1-integrin receptor for fibronectin in human kidney glomeruli. Am J Pathol 134: 481, 1989

Kern PA, Knedler A, Eckel RH: Isolation and culture of microvascular endothelium from human adipose tissue. J Clin Invest 71: 1822, 1983

Kiefel V, Santoso S, Katzmann B, Mueller-Eckhardt C: The Br^a/Br^b alloantigen system on human platelets. Blood 73: 2219, 1989

Kimani G, Tonneson MG, Henson PM: Stimulation of eosinophil adherence to human vascular endothelial cells in vitro by platelet-activating factor. J Immunol 140: 3161, 1988

Kirkpatrick CJ, Bultmann BD, Gruler H: Interaction between enteroviruses and human endothelial cells in vitro. Alterations in the physical properties of endothelial cell plasma membrane and adhesion of human granulocytes. Am J Pathol 118: 15, 1985

Kishimoto TK, O'Connor K, Lee A, Roberts TM, Springer TA: Cloning of the β-subunit of the leucocyte adhesion proteins: homology to an extracellular matrix receptor defines a novel supergene family. Cell 48: 681, 1987a

Kishimoto TK, Hollander N, Roberts TM, Anderson DC, Springer TA: Heterogeneous mutations in the β-subunit common to the LFA-1, Mac-1, and p150,95 glycoproteins cause leukocyte adhesion deficiency. Cell 50: 193, 1987b

Kishimoto TK, Jutila MA, Berg EL, Butcher EC: Neutrophil Mac-1 and MEL-14 adhesion proteins inversely regulated by chemotactic factors. Science 245: 1238, 1989

Kishimoto TK, Jutila MA, Butcher EC: Identification of a human peripheral lymph node homing receptor: A rapidly down-regulated adhesion molecule. Proc Natl Acad Sci USA 87: 2244, 1990

Kissmeyer-Nielsen F, Olsen S, Petersen VP, Fjeldborg O: Hyperacute rejection of kidney allografts, associated with pre-existing humoral antibodies against donor cells. Lancet 2: 662, 1966

Kissmeyer-Nielsen F, Svejgaard A, Hauge M: Genetics of the human HL-A transplantation system. Nature 219: 1116, 1968

Klein LM, Larker RM, Matis WL, Murphy GF: Degranulation of human mast cells induces an endothelial antigen central to leukocyte adhesion. Proc Natl Acad Sci USA 86: 8972, 1989

Kloczewiak M, Timmons S, Lukas TJ, Hawiger J: Platelet receptor recognition site on human fibrinogen. Synthesis and structure-function relationship of peptides corresponding to the carboxy-terminal segment of the γ chain. Biochemistry 23: 1767, 1984

Knudsen KA, Horwitz AF, Buck CA: A monoclonal antibody identifies a glycoprotein complex involved in cell-substratum adhesion. Exp Cell Res 157: 218, 1985

Knudtzon S, Mortensen BT: Growth stimulation of human bone marrow cells in agar culture by vascular cells. Blood 46: 937, 1975

Kohler G: The technique of hybridoma production. IN: Immunological Methods II. Ed: Lefkovits I, Pervis B (Academic Press): 285, 1981

Koide S, Steinman RM: Induction of murine interleukin 1: stimuli and responsive primary cells. Proc Natl Acad Sci USA 84: 3802, 1987

van Kooyk Y, Kemenade PVDW, Weder P, Kuijpers TW, Figdor CG: Enhancement of LFA-1-mediated cell adhesion by triggering through CD2 or CD3 on T lymphocytes. Nature 342: 811, 1989

Koretz K, Momburg F, Otto HF, Moller P: Sequential induction of MHC antigens on autochthonous cells of the ileum affected by Crohn's disease. Am J Pathol 129: 493, 1987

Korhonen M, Ylanne J, Laitinen L, Virtanen I: Distribution of β_1 and β_3 integrins in human fetal and adult kidney. Lab Invest 62: 616, 1990a

Korhonen M, Ylanne J, Laitinen L, Virtanen I: The α_1-α_6 subunits of integrins are characteristically expressed in distinct segments of developing and adult human nephron. J Cell Biol 111: 1245, 1990b

Kraal G, Duijvestijn AM, Hendriks HH: The endothelium of the high endothelial venule: a specialized endothelium with unique properties. Exp Cell Biol 55: 1, 1987

Kramer P, Bijnen AB, ten Kate FW, Jeekel J, Weimar W: Recombinant leucocyte interferon A induces steroid-resistant acute vascular rejection episodes in renal transplant recipients. Lancet 1: 989, 1984

Kramer RH, Gonzalez R, Nicolson GL: Metastatic tumor cells adhere preferentially to the extracellular matrix underlying vascular endothelial cells. Int J Cancer 26: 639, 1980

Kramer RH, Marks N: Identification of integrin collagen receptors on human melanoma cells. J Biol Chem 264: 4684, 1989a

Kramer RH, McDonald KA, Vu MP: Human melanoma cells express a novel integrin receptor for laminin. J Biol Chem 264: 15642, 1989b

Krensky AM, Sanchez-Madrid F, Robbins E, Nagy JA, Springer TA, Burakoff SJ: The functional significance, distribution, and structure of LFA-1, LFA-2, and LFA-3: cell surface antigens associated with CTL-target interactions. J Immunol 131: 611, 1983

Krensky AM, Robbins E, Springer TA, Burakoff SJ: LFA-1, LFA-2, and LFA-3 antigens are involved in CTL-target conjugation. J Immunol 132: 2180, 1984

Krensky AM, Weiss A, Crabtree G, Davis MM, Parham P: T lymphocyte-antigen interactions in transplant rejection. NEJM 322: 510, 1990

Krissansen GW, Elliott MJ, Lucas CM, Stomski FC, Berndt MC, Cheresh DA, Lopez AF, Burns GF: Identification of a novel integrin β subunit expressed on cultured monocytes (macrophages). Evidence that one α subunit can associate with multiple β subunits. J Biol Chem 265: 823, 1990

Kuboto Y, Kleinman HK, Martin GR, Lowley TJ: Role of laminin and basement membrane in the morphological differentiation of human endothelial cells into capillary-like structures. J Cell Biol 107: 1589, 1988

Kumamoto K, Stewart TA, Johnson AR, Erdos EG: Prolylcarboxypeptidase (angiotensinase C) in human lung and cultured cells. J Clin Invest 67: 210, 1981

Kunicki TJ, Nugent DJ, Staats SJ, Orchekowski RP, Wayner EA, Carter WG: The human fibroblast class II extracellular matrix receptor mediates platelet adhesion to collagen and is identical to the platelet glycoprotein Ia-IIa complex. J Biol Chem 263: 4516, 1988

van Kuppevelt THMSM, Languino LR, Gailit JO, Suzuki S, Ruoslahti E: An alternative cytoplasmic domain of the integrin β_3 subunit. Proc Natl Acad Sci USA 86: 5415, 1989

Kuppner MC, van Meir E, Hamou MF, de Tribolet N: Cytokine regulation of intercellular adhesion molecule-1 (ICAM-1) expression on human glioblastoma cells. Clin Exp Immunol 81: 142, 1990

Kurki P, Vartio T, Virtanen I: Mitogen stimulation promotes human T lymphocyte adhesion to fibronectin. Scand J Immunol 26: 645, 1987

Kurnick JT, Preffer FI, Colvin RB, Leary CP, Boyle L: Functional analysis of lymphocyte subsets and clones infiltrating renal allografts. Trans Proc 19: 394, 1987

Kurt-Jones EA, Fiers W, Pober JS: Membrane interleukin 1 induction on human endothelial cells and dermal fibroblasts. J Immunol 139: 2317, 1987

Kuypers TW, Roos D: Leukocyte membrane adhesion proteins LFA-1, CR3, and p150,95: a review of functional and regulatory aspects. Res Immunol 140: 461, 1989

Lafferty KJ, Cooley MA, Woolnough J, Walker KZ: Thyroid allograft immunogenicity is reduced after a period in organ culture. Science 188: 259, 1975

Lafferty KJ, Prowse SJ, Simeonovic CJ, Warren HS: Immunobiology of tissue transplantation: a return to the passenger leukocyte concept. Ann Rev Immunol 1: 143, 1983

Lam SCT, Plow EF, Smith MA, Andrieux A, Ryckwaert JJ, Marguerie G, Ginsberg MH: Evidence that arginyl-glycyl-aspartate peptides and fibrinogen γ chain peptides share a common binding site on platelets. J Biol Chem 262: 947, 1987

Lam SCT, Plow EF, D'Souza SE, Cheresh DA, Frelinger AL, Ginsberg MH: Isolation and characterisation of a platelet membrane protein related to the vitronectin receptor. J Biol Chem 264: 3742, 1989

Lamas AM, Mulroney CM, Schleimer RP: Studies on the adhesive interaction between purified human eosinophils and cultured vascular endothelial cells. J Immunol 140: 1500, 1988

Lampert IA, Suitters AJ, Chisholm PM: Expression of Ia antigen on epidermal keratinocytes in graft-versus-host disease. Nature 293: 149, 1981

Languino LR, Gehlsen KR, Wayner EA, Carter WG, Engvall E, Ruoslahti E: Endothelial cells use $\alpha_2\beta_1$ integrin as a laminin receptor. J Cell Biol 109: 2455, 1989

Larsen E, Celi A, Gilbert GE, Furie BC, Erban JK, Bonfanti R, Wagner DD, Furie B: PADGEM protein: a receptor that mediates the interaction of activated platelets with neutrophils and monocytes. Cell 59: 305, 1989

Lasky LA, Singer MS, Yednock TA, Dowbenko D, Fennie C, Rodriguez H, Nguyen T, Stachel S, Rosen SD: Cloning of a lymphocyte homing receptor reveals a lectin domain. Cell 56: 1045, 1989

Lassila O, Vainio O, Matzinger P: Can B cells turn on virgin T cells? Nature 334: 253, 1988

Law SKA, Gagnon J, Hildreth JEK, Wells CE, Willis AC, Wong AJ: The primary structure of the β-subunit of the cell surface adhesion glycoproteins LFA-1, CR3, and p150,95 and its relationship to the fibronectin receptor. EMBO J 6: 915, 1987

Lawler J, Hynes RO: An integrin receptor on normal and thrombasthenic platelets that binds thrombospondin. Blood 74: 2022, 1989

Lawrence MB, Smith CW, Eskin SG, McIntire LV: Effect of venous sheer stress on CD18-mediated neutrophil adhesion to cultured endothelium. Blood 75: 227, 1990

Le PT, Vollger LW, Haynes BF, Singer KH: Ligand binding to the LFA-3 cell adhesion molecule induces IL-1 production by human thymic epithelial cells. J Immunol 144: 4541, 1990

Leeksma OC, Zandbergen-Spaargaren J, Giltay JC, van Mourik JA: Cultured human endothelial cells synthesise a plasma membrane protein complex immunologically related to the platelet glycoprotein IIb/IIIa complex. Blood 67: 1176, 1986

Leewenberg JFM, von Asmuth EJU, Jeunhomme TMAA, Buurman WA: IFN-γ regulates the expression of the adhesion molecule ELAM-1 and IL-6 production by human endothelial cells in vitro. J Immunol 145: 2110, 1990

Leppink DM, Bishop DK, Sedmak DD, Henry ML, Ferguson RM, Streeter PR, Butcher EC, Orosz CG: Inducible expression of an endothelial cell antigen on murine myocardial vasculature in association with interstitial cellular infiltration. Transplantation 48: 874, 1989

Leptin M, Aebersold R, Wilcox M: Drosophila position-specific antigens resemble the vertebrate fibronectin-receptor family. EMBO J 6: 1037, 1987

Leptin M, Bogaert T, Lehmann R, Wilcox M: The function of PS integrins during Drosophila embryogenesis. Cell 56: 401, 1989

Leung DYM, Collins T, Lapierre LA, Geha RS, Pober JS: Immunoglobulin M antibodies present in the acute phase of Kawasaki syndrome lyse cultured vascular endothelial cells stimulated by gamma interferon. J Clin Invest 77: 1428, 1986a

Leung DYM, Geha RS, Newburger JW, Burns JC, Fiers W, Lapierre LA, Pober JS: Two monokines, interleukin 1 and tumor necrosis factor, render cultured vascular endothelial cells susceptible to lysis by antibodies circulating during Kawasaki syndrome. J Exp Med 164: 1958, 1986b

Leung DYM, Havens PL, Moake JL, Kim M, Pober JS: Lytic anti-endothelial cell antibodies in haemolytic-uraemic syndrome. Lancet 2: 183, 1988

Leung DYM, Kurt-Jones E, Newburger JW, Cotran RS, Burns JC, Pober JS: Endothelial cell activation and high interleukin-1 secretion in the pathogenesis of acute Kawasaki disease. Lancet 2: 1298, 1989

Levin EG, Merzec U, Anderson J, Harker LA: Thrombin stimulates tissue plasminogen activator release from cultured human endothelial cells. J Clin Invest 74: 1988, 1984

Levine JD, Harlan JM, Harker LA, Joseph ML, Counts RB: Thrombin-mediated release of factor VIII antigen from human umbilical vein endothelial cells in culture. Blood 60: 531, 1982

Lewinsohn DM, Bargatze RF, Butcher EC: Leukocyte-endothelial cell recognition: evidence of a common molecular mechanism shared by neutrophils, lymphocytes, and other leukocytes. J Immunol 138: 4313, 1987

Lewis MS, Whatley RE, Cain P, McIntyre TM, Prescott SM, Zimmerman GA: Hydrogen peroxide stimulates the synthesis of platelet-activating factor by endothelium and induces endothelial-cell dependent neutrophil adhesion. J Clin Invest 82: 2045, 1988

Libby P, Ordovas JM, Auger KR, Robbins AH, Birinyi LK, Dinarello CA: Endotoxin and tumor necrosis factor induce interleukin-1 gene expression in adult human vascular endothelial cells. Am J Pathol 124: 179, 1986

Liotta LA: Tumor invasion and metastases - role of the extracellular matrix. Cancer Res 46: 1, 1986

Lisby S, Ralfkiaer E, Rothlein R, Vejlsgaard GL: Intercellular adhesion molecule-1 (ICAM-1) expression correlated to inflammation. Br J Dermatol 120: 479, 1989

Lo SK, van Seventer GA, Levin SM, Wright SD: Two leukocyte receptors (CD11a/CD18 and CD11b/CD18) mediate transient adhesion to endothelium by binding to different ligands. J Immunol 143: 3325, 1989

Locksley RM, Heinzel FP, Shepard HM, Agosti J, Eessalu TE, Aggarwal BB, Harlan JM: Tumor necrosis factors α and β differ in their capacities to generate interleukin 1 release from human endothelial cells. J Immunol 139: 1891, 1987

Loftus JC, O'Toole TE, Plow EF, Glass A, Frelinger AL, Ginsberg MH: A β_3 integrin mutation abolishes ligand binding and alters divalent cation-dependent conformation. Science 249: 915, 1990

Lollar P, Owen WG: Clearance of thrombin from circulation in rabbits by high-affinity binding sites on endothelium. Possible role in the inactivation of thrombin by antithrombin III. J Clin Invest 66: 1222, 1980

Loskutoff DJ, Mussoni L: Interactions between fibrin and the plasminogen activators produced by cultured endothelial cells. Blood 62: 62, 1983

Loveland BE, McKenzie IFC: Which T cells cause graft rejection? Transplantation 33: 217, 1982

Lucas MG, Green AM, Telen MJ: Characterization of the serum In(Lu)-related antigen: identification of a serum protein related to erythrocyte p80. Blood 73: 596, 1989

Luscinkas FW, Brock AF, Arnaout MA, Gimbrone MA: Endothelial-leukocyte adhesion molecule-1-dependent and leukocyte (CD11/CD18)-dependent mechanisms contribute to polymorphonuclear leukocyte adhesion to cytokine-activated human vascular endothelium. J Immunol 142: 2257, 1989

MacGregor RR, Friedman HM, Macarak EJ, Kefalides NA: Virus infection of endothelial cells increases granulocyte adherence. J Clin Invest 65: 1469, 1980

Maciag T, Cerundolo J, Ilsley S, Kelley PR, Forand R: An endothelial cell growth factor from bovine hypothalamus: identification and partial characterization. Proc Natl Acad Sci USA 76: 5674, 1979

Maciag T, Kadish J, Wilkins L, Stemerman MB, Weinstein R: Organisational behaviour of human umbilical vein endothelial cells. J Cell Biol 94: 511, 1982

Maciag T, Mehlman T, Friesen R: Heparin binds endothelial cell growth factor, the principal endothelial cell mitogen in bovine brain. Science 225: 932, 1984

MacKrell AJ, Blumberg B, Haynes SR, Fesster JH: The lethal myospheroid gene of Drosophila encodes a membrane protein homologous to vertebrate integrin β subunits. Proc Natl Acad Sci USA 85: 2633, 1988

Madri JA, Pratt BM, Tucker AM: Phenotypic modulation of endothelial cells by transforming growth factor-β depends upon the composition and organisation of the extracellular matrix. J Cell Biol 106: 1375, 1988

Maeda T, Oyama R, Ichihara-Tanaka K, Kimizuka F, Kato I, Titani K, Sekiguchi K: A novel cell adhesive protein engineered by insertion of the Arg-Gly-Asp-Se tetrapeptide. J Biol Chem 264: 15165, 1989

Maio M, Tessitori G, Pinto A, Temponi M, Colombatti A, Ferrone S: Differential role of distinct determinants of intercellular adhesion molecule-1 in immunologic phenomena. J Immunol 143: 181, 1989

Makgoba MW, Shaw S, Gugel EA, Sanders ME: Human T cell rosetting is mediated by LFA-3 on autologous erythrocytes. J Immunol 138: 3587, 1987

Makgoba MW, Sanders ME, Luce GEG, Dustin ML, Springer TA, Clark EA, Mannoni P, Shaw S: ICAM-1, a ligand for LFA-1-dependent adhesion of B, T, and myeloid cells. Nature 331: 86, 1988a

Makgoba MW, Sanders ME, Luce GEG, Gugel EA, Dustin ML, Springer TA, Shaw S: Functional evidence that intercellular adhesion molecule-1 (ICAM-1) is a ligand for LFA-1-dependent adhesion in T cell-mediated cytotoxicity. Eur J Immunol 18: 637, 1988b

Marcantonio EE, Hynes RO: Antibodies to the conserved cytoplasmic domain of the integrin β_1 subunit react with proteins in vertebrates, invertebrates, and fungi. J Cell Biol 106: 1765, 1988

Marcum JA, Rosenberg RD: Role of endothelial cell surface heparin-like polysaccharides. Annals New York Academy of Sciences 556: 81, 1989

Margulies DH: Naked or peptide-clothed MHC? Nature 342: 124, 1989

Marks RM, Todd RF, Ward PA: Rapid induction of neutrophil-endothelial adhesion by endothelial complement fixation. Nature 339: 314, 1989

Marlin SD, Springer TA: Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen 1 (LFA-1). Cell 51: 813, 1987

Marlin SD, Staunton DE, Springer TA, Stratowa C, Sommergruber W, Merluzzi VJ: A soluble form of intercellular adhesion molecule-1 inhibits rhinovirus infection. Nature 344: 70, 1990

Marrack P, Kappler J: The T cell receptor. Science 238: 1073, 1987

Martz E: LFA-1 and other accessory molecules functioning in adhesions of T and B lymphocytes. Human Immunology 18: 3, 1987

Mason DW, Morris PJ: Effector mechanisms in allograft rejection. Ann Rev Immunol 4: 119, 1986

Masuyama J, Minato N, Kano S: Mechanisms of lymphocyte adhesion to human vascular endothelial cells in culture. T lymphocyte adhesion to endothelial cells through endothelial HLA-DR antigens induced by gamma interferon. J Clin Invest 77: 1596, 1986

Matsubara T, Ziff M: Increased superoxide anion release from human endothelial cells in repsonse to cytokines. J Immunol 137: 3295, 1986

Matsuyama T, Yamada A, Kay J, Yamada KM, Akiyama SK, Schlossman SF, Morimoto C: Activation of CD4 cells by fibronectin and anti-CD3 antibody. A synergistic effect mediated by the VLA-5 fibronectin receptor complex. J Exp Med 170: 1133, 1989

Matzinger P, Bevan MJ: Why do so many lymphocytes respond to major histocompatibility antigens? Cell Immunol 29: 1, 1977

Maxfield SR, Moulder K, Koning F, Elbe A, Stingl G, Coligan JE, Shevach EM, Yokoyama WM: Murine T cells express a cell surface receptor for multiple extracellular matrix proteins. Identification and characterization with monoclonal antibodies. J Exp Med 169: 2173, 1989

Mayrhofer G, Schon-Hegrad MA: Ia antigens in rat kidney, with special reference to their expression in tubular epithelium. J Exp Med 157: 2097, 1983

Mazerolles F, Lumbroso C, Lecomte O, Le Deist F, Fischer A: The role of lymphocyte function-associated antigen 1 (LFA-1) in the adherence of T lymphocytes to B lymphocytes. Eur J Immunol 18: 1229, 1988

McCarron RM, Spatz M, Kempski O, Hogan RN, Muehl L, McFarlin DE: Interaction between myelin basic protein-sensitised T lymphocytes and murine cerebral vascular endothelial cells. J Immunol 137: 3428, 1986

McEver RP, Martin MN: A monoclonal antibody to a membrane glycoprotein binds only to activated platelets. J Biol Chem 259: 9799, 1984

McEver RP, Beckstead JH, Moore KL, Marshall-Carlson L, Bainton DF: GMP-140, a platelet α-granule membrane protein, is also synthesised by vascular endothelial cells and is localised in Weibel-Palade bodies. J Clin Invest 84: 92, 1989

McIntyre BW, Evans EL, Bednarczyk JL: Lymphocyte surface antigen L25 is a member of the integrin receptor superfamily. J Biol Chem 264: 13745, 1989

McIntyre TM, Zimmerman GA, Prescott SM: Leukotrienes C_4 and D_4 stimulate human endothelial cells to synthesize platelet-activating factor and bind neutrophils. Proc Natl Acad Sci USA 83: 2204, 1986

McLean JW, Vestal DJ, Cheresh DA, Bodary SC: cDNA sequence of the human integrin β_5 subunit. J Biol Chem 265: 17126, 1990

Mecham RP, Madaras J, McDonald JA, Ryan U: Elastin production by cultured calf pulmonary artery endothelial cells. J Cell Physiol 116: 282, 1983

Mendelsohn CL, Wimmer E, Racaniello VR: Cellular receptor for Poliovirus: molecular cloning, nucleotide sequence, and expression of a new member of the immunoglobulin superfamily. Cell 56: 855, 1989

Menko AS, Boettiger D: Occupation of the extracellular matrix receptor, integrin, is a control point for myogenic differentiation. Cell 51: 51, 1987

Mentzer SJ, Gromkowski SH, Krensky AM, Burakoff SJ, Martz E: LFA-1 membrane molecule in the regulation of homotypic adhesions of human B lymphocytes. J Immunol 135: 9, 1985

Mentzer SJ, Burakoff SJ, Faller DV: Adhesion of T lymphocytes to human endothelial cells is regulated by the LFA-1 membrane molecule. J Cell Physiol 126: 285, 1986a

Mentzer SJ, Krensky AM, Burakoff SJ: Mapping functional epitopes of the human LFA-1 glycoprotein: monoclonal antibody inhibition of NK and CTL effectors. Human Immunology 17: 288, 1986b

Mentzer SJ, Crimmins MAV, Burakoff SJ, Faller DV: Alpha and beta subunits of the LFA-1 membrane molecule are involved in human monocyte-endothelial cell adhesion. J Cell Physiol 130: 410, 1987

Messadi DV, Pober JS, Fiers W, Gimbrone MA, Murphy GF: Induction of an activation antigen on postcapillary venular endothelium in human skin organ culture. J Immunol 139: 1557, 1987

Messer-Peters P, Kamarck ME, Hemler ME, Strominger JL, Ruddle FH: Genetic and biochemical characterization of human lymphocyte cell surface antigens. The A-1A5 and A3A4 determinants. J Exp Med 159: 1441, 1984

Meuer SC, Hussey RE, Fabbi M, Fox D, Acuto O, Fitzgerald KA, Hodgdon JC, Protentis SF, Reinherz EL: An alternative pathway of T-cell activation: functional role for the 50 kD T11 sheep erythrocyte receptor protein. Cell 36: 897, 1984

Meyer D, Baumgartner HR: Role of von Willebrand factor in platelet adhesion to the subendothelium. Br J Haematol 54: 1, 1983

Miles LA, Levin EG, Plescia J, Collen D, Plow EF: Plasminogen receptors, urokinase receptors, and their modulation on human endothelial cells. Blood 72: 628, 1988

Miller LH: Malaria. Binding of infected red cells. Nature 341: 18, 1989

Miller LJ, Schwarting R, Springer TA: Regulated expression of the Mac-1, LFA-1, p150,95 glycoprotein family during leukocyte differentiation. J Immunol 137: 2891, 1986

Miller LJ, Springer TA: Biosynthesis and glycosylation of p150,95 and related leukocyte adhesion proteins. J Immunol 139: 842, 1987a

Miller LJ, Bainton DF, Borregaard N, Springer TA: Stimulated mobilization of monocyte Mac-1 and p150,95 adhesion proteins from intracellular vesicular compartment to the cell surface. J Clin Invest 80: 535, 1987b

Miltenburg AMM, Meijer-Paape ME, Weening JJ, Daha MR, van Es LA, van der Woude FJ: Induction of antibody-dependent cellular cytotoxicity against endothelial cells by renal transplantation. Transplantation 48: 681, 1989

Miossec P, Cavender D, Ziff M: Production of interleukin 1 by human endothelial cells. J Immunol 136: 2486, 1986a

Miossec P, Ziff M: Immune interferon enhances the production of interleukin 1 by human endothelial cells stimulated with lipopolysaccharide. J Immunol 137: 2848, 1986b

Mishra GC, Berton MT, Oliver KG, Krammer PH, Uhr JW, Vitetta ES: A monoclonal anti-mouse LFA-1α antibody mimics the biological effects of B cell stimulatory factor-1 (BSF-1). J Immunol 137: 1590, 1986

Miyake K, Underhill CB, Lesley J, Kincade PW: Hyaluronate can function as a cell adhesion molecule and CD44 participates in hyaluronate recognition. J Exp Med 172: 69, 1990

Modlin RL, Melancon-Kaplan J, Young SMM, Pirmez C, Kino H, Convit J, Rea TH, Bloom BR: Learning from lesions: patterns of tissue inflammation in leprosy. Proc Natl Acad Sci USA 85: 1213, 1988

Moingeon P, Chang HC, Wallner BP, Stebbins C, Frey AZ, Reinherz EL: CD2-mediated adhesion facilitates T lymphocyte antigen recognition function. Nature 339: 312, 1989

Montesano R, Orci L, Vassalli P: In vitro rapid organization of endothelial cells into capillary-like networks is promoted by collagen matrices. J Cell Biol 97: 1648, 1983

Moraes JR, Stastny P: A new antigen system expressed in human endothelial cells. J Clin Invest 60: 449, 1977

Morimoto C, Letvin NL, Boyd AW, Hagan M, Brown HM, Kornecki MM, Schlossman SF: The isolation and characterization of the human helper inducer T cell subset. J Immunol 134: 3762, 1985

Morimoto C, Rudd CE, Letvin NL, Schlossman SF: A novel epitope of the LFA-1 antigen which can distinguish killer effector and suppressor cells in human CD8 cells. Nature 330: 479, 1987

Moser R, Schleiffenbaum B, Groscurth P, Fehr J: Interleukin 1 and tumor necrosis factor stimulate human vascular endothelial cells to promote transendothelial neutrophil passage. J Clin Invest 83: 444, 1989

Mosher DF, Doyle MJ, Jaffe EA: Synthesis and secretion of thrombospondin by cultured human endothelial cells. J Cell Biol 93: 343, 1982

Mosher DF: Physiology of fibronectin. Ann Rev Med 35: 561, 1984

Mould AP, Wheldon LA, Komoriya A, Wayner EA, Yamada KM, Humphries MJ: Affinity chromatographic isolation of the melanoma adhesion receptor for the IIICS region of fibronectin and its identification as the integrin $\alpha_4\beta_1$. J Biol Chem 265: 4020, 1990

Mountz JD, Gause WC, Finkelman FD, Steinberg AD: Prevention of lymphadenopathy in MRL-lpr/lpr mice by blocking peripheral lymph node homing with MEL-14 in vivo. J Immunol 140: 2943, 1988

Mourad W, Geha RS, Chatila T: Engagement of major histocompatibility complex class II molecules induces sustained, lymphocyte function-associated molecule 1-dependent cell adhesion. J Exp Med 172: 1513, 1990

van Mourik JA, Leeksma OC, Reinders JH, de Groot PG, Zandergen-Spaargaren J: Vascular endothelial cells synthesise a plasma membrane protein indistinguishable from the platelet membrane glycoprotein IIa. J Biol Chem 260: 11300, 1985

Mueller SN, Rosen EM, Levine EM: Cellular senescence in a cloned strain of bovine fetal aortic endothelial cells. Science 207: 889, 1980

Muller CA, Markovic-Lipkovski J, Risler T, Bohle A, Muller GA: Expression of HLA-DQ, -DR, and -DP antigens in normal kidney and glomerulonephritis. Kidney International 35: 116, 1989

Muller G, Behrens J, Nussbaumer U, Bohlen P, Birchmeier W: Inhibitory action of transforming growth factor β on endothelial cells. Proc Natl Acad Sci USA 84: 5600, 1987

Munro JM, Pober JS, Cotran RS: Tumor necrosis factor and interferon-γ induce distinct patterns of endothelial activation and associated leukocyte accumulation in skin of Papio Anubis. Am J Path 135: 121, 1989

Myones BL, Dalzell JG, Hogg N, Ross GD: Neutrophil and monocyte cell surface p150,95 has iC3b-receptor (CR4) activity resembling CR3. J Clin Invest 82: 640, 1988

Nachman RL, Hajjar KA, Silverstein RL, Dinarello CA: Interleukin 1 induces endothelial cell synthesis of plasminogen activator inhibitor. J Exp Med 163: 1595, 1986

Naidet C, Semeriva M, Yamada KM, Thiery JP: Peptides containing the cell-attachment recognition signal Arg-Gly-Asp prevent gastrulation in Drosophila embryos. Nature 325: 348, 1987

Nakache M, Berg EL, Streeter PR, Butcher EC: The mucosal vascular addressin is a tissue-specific endothelial cell adhesion molecule for circulating lymphocytes. Nature 337: 179, 1989

Naparstek Y, Cohen IR, Fuks Z, Vlodavsky I: Activated T lymphocytes produce a matrix-degrading heparan sulphate endoglycosidase. Nature 310: 241, 1984

Nathan C, Srimal S, Farber C, Sanchez E, Kabbash L, Asch A, Gailit J, Wright SD: Cytokine-induced respiratory burst of human neutrophils: dependence on extracellular matrix proteins and CD11/CD18 integrins. J Cell Biol 109: 1341, 1989

Navarro RF, Jalkanen ST, Hsu M, Soenderstrup-Hansen G, Goronzy J, Weyand C, Fathman CG, Clayberger C, Krensky AM, Butcher EC: Human T cell clones express functional homing receptors required for normal lymphocyte trafficking. J Exp Med 162: 1075, 1985

Nawroth PP, Stern DM: Modulation of endothelial cell hemostatic properties by tumor necrosis factor. J Exp Med 163: 740, 1986

Neff NT, Lowrey C, Decker C, Tovar A, Damsky C, Buck C, Horwitz AF: A monoclonal antibody detaches embryonic skeletal muscle from extracellular matrices. J Cell Biol 95: 654, 1982

Neppert J, Mueller-Eckhardt G, Stranzenbach W, Gottsche B, Mueller-Eckhardt C: Endothelial monocyte antigens are coded for by loci centromeric of HLA-B. Vox Sang 49: 221, 1985

Nickoloff BJ: Role of interferon-γ in cutaneous trafficking of lymphocytes with emphasis on molecular and cellular adhesion events. Arch Dermatol 124: 1835, 1988

Nieuwenhuis HK, Akkerman JWN, Handijk WPW, Sixma JJ: Human blood platelets showing no response to collagen fail to express surface glycoprotein Ia. Nature 318: 470, 1985

van Noesel C, Miedma F, Brouwer M, de Rie MA, Aarden LA, van Lier RAW: Regulatory properties of LFA-1 α and β chains in human T-lymphocyte activation. Nature 333: 850, 1988

Noll H, Matranga V, Cervello M, Humphreys T, Kuwasaki B, Adelson D: Characterization of toposomes from sea urchin blastula cells: a cell organelle mediating cell adhesion and expressing positional information. Proc Natl Acad Sci USA 82: 8062, 1985

Nottenburg C, Rees G, St John T: Isolation of mouse CD44 cDNA: structural features are distinct from the primate cDNA. Proc Natl Acad Sci USA 86: 8521, 1989

Nuchtern JG, Biddison WE, Klausner RD: Class II MHC molecules can use the endogenous pathway of antigen presentation. Nature 343: 74, 1990

Nunez G, Ball EJ, Stastny P: Accessory cell function of human endothelial cells. 1. A subpopulation of Ia positive cells is required for antigen presentation. J Immunol 131: 666, 1983

Obara M, Kang MS, Yamada KM: Site-directed mutagenesis of the cell-binding domain of human fibronectin: separable, synergistic sites mediate adhesive function. Cell 53: 649, 1988

O'Connell KA, Edidin M: A mouse lymphoid endothelial cell line immortalized by simian virus 40 binds lymphocytes and retains functional characteristics of normal endothelial cells. J Immunol 144: 521, 1990

Ockenhouse CF, Tandon NN, Magowan C, Jamieson GA, Chulay JD: Identification of a platelet membrane glycoprotein as a falciparum malaria sequestration receptor. Science 243: 1469, 1989

O'Farrell P: High resolution two-dimensional electrophoresis of proteins. J Biol Chem 250: 4007, 1975

O'Flaherty JT, Craddock PR, Jacob HS: Effect of intravascular complement activation on granulocyte adhesiveness and distribution. Blood 51: 731, 1978

O'Halloran T, Beckerle MC, Burridge K: Identification of talin as a major cytoplasmic protein implicated in platelet activation. Nature 317: 449, 1985

Oldberg A, Franzen A, Heinegard D: Cloning and sequence analysis of rat bone sialoprotein (osteopontin) cDNA reveals an Arg-Gly-Asp cell-binding sequence. Proc Natl Acad Sci USA 83: 8819, 1986

Oppenheimer-Marks N, Ziff M: Binding of normal human mononuclear cells to blood vessels in rheumatoid arthritis synovial membrane. Arth Rheum 29: 789, 1986

Oppenheimer-Marks N, Davis LS, Lipsky PE: Human T lymphocyte adhesion to endothelial cells and transendothelial migration. Alteration of receptors use relates to the activation status of both the T cell and the endothelial cell. J Immunol 145: 140, 1990

Oquendo P, Hundt E, Lawler J, Seed B: CD36 directly mediates cytoadherence of Plasmodium falciparum parasitized erythrocytes. Cell 58: 95, 1989

Osborn L, Hession C, Tizard R, Vassallo C, Luhowskyj S, Chi-Rosso G, Lob R: Direct expression cloning of vascular cell adhesion molecule 1, a cytokine-induced endothelial protein that binds to lymphocytes. Cell 59: 1203, 1989

O'Toole TE, Loftus JC, Plow EF, Glass AA, Harper JR, Ginsberg MH: Efficient surface expression of platelet GP IIb-IIIa requires both subunits. Blood 74: 14, 1989

O'Toole TE, Loftus JC, Du X, Glass AA, Ruggeri ZM, Shattil SJ, Plow EF, Ginsberg MH: Affinitiy modulation of the $\alpha_{IIb}\beta_3$ integrin (platelet GPIIb-IIIa) is an intrinsic property of the receptor. Cell Regulation (in press)

Ouaissi MA, Cornette J, Afchain D, Capron A, Gras-Masse H, Tartar A: Trypanosoma cruzi infection inhibited by peptides modeled from a fibronectin cell attachment domain. Science 234: 603, 1986

Painter RG, Prodonz KN, Gaarde W: Isolation of a subpopulation of glycoprotein IIb-IIIa from platelet membranes that is bound to membrane actin. J Cell Biol 100: 652, 1985

Palmer RMJ, Ferrige AG, Moncoda S: Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 327: 524, 1987

Pals ST, Kraal G, Horst E, de Groot A, Scheper RJ, Meijer CJLM: Human lymphocyte-high endothelial venule interaction: organ-selective binding of T and B lymphocyte populations to high endothelium. J Immunol 137: 760, 1986

Pals ST, den Otter A, Miedema F, Kabel P, Keizer GD, Scheper RJ, Meijer CJLM: Evidence that leukocyte function-associated antigen-1 is involved in recirculation and homing of human lymphocytes via high endothelial venules. J Immunol 140: 1851, 1988

Pals ST, Horst E, Ossekoppele GJ, Figdor CG, Scheper RJ, Meijer CJLM: Expression of lymphocyte homing receptor as a mechanism of dissemination in non-Hodgkin's lymphoma. Blood 73: 885, 1989a

Pals ST, Hogervorst F, Keizer GD, Thepen T, Horst E, Figdor CG: Identification of a widely distributed 90 kDa glycoprotein that is homologous to the Hermes-1 human lymphocyte homing receptor. J Immunol 143: 851, 1989b

Pantaleo G, Olive D, Poggi A, Kozumbo WJ, Moretta L, Moretta A: Transmembrane signalling via the T11-dependent pathway of human T cell activation. Evidence for the involvement of 1,2-diacylglycerol and inositol phosphates. Eur J Immunol 17: 55, 1987

Pardi R, Bender JR, Dettori C, Giannazza E, Engleman EG: Heterogeneous distribution and transmembrane signalling properties of lymphocyte function-associated antigen (LFA-1) in human lymphocyte subsets. J Immunol 143: 3157, 1989

Patarroyo M, Biberfeld P, Klein E, Klein G: Phorbol 12,13-dibutyrate ($P(Bu)_2$)-treated human blood mononuclear cells bind to each other. Cell Immunol 75: 144, 1983a

Patarroyo M, Jondal M, Gordon J, Klein E: Characterisation of the phorbol 12,13-dibutyrate ($P(Bu)_2$) induced binding between human lymphocytes. Cell Immunol 81: 373, 1983b

Patarroyo M, Gahmberg CG: Phorbol 12,13-dibutyrate enhances lateral redistribution of membrane glycoproteins in human blood lymphocytes. Eur J Immunol 14: 781, 1984

Patarroyo M, Beatty PG, Fabre JW, Gahmberg CG: Identification of a cell surface protein complex mediating phorbol ester-induced adhesion (binding) among human mononuclear leukocytes. Scand J Immunol 22: 171, 1985

Patarroyo M, Clark EA, Prieto J, Kantor C, Gahmberg CG: Identification of a novel adhesion molecule in human leukocytes by monoclonal antibody LB-2. FEBS Letters 210: 127, 1987

Paul LC, van Es LA, Kalff MW, de Graeff J: Intrarenal distribution of endothelial antigens recognised by antibodies from renal allograft recipients. Trans Proc 11: 427, 1979a

Paul LC, van Es LA, van Rood JJ, van Leeuwen A, de la Riviere GB, de Graeff J: Antibodies directed against antigens on the endothelium of peritubular capillaries in patients with rejecting renal allografts. Transplantation 27: 175, 1979b

Paul LC, Claas FHJ, van Es LA, Kalff MW, de Graeff J: Accelerated rejection of a renal allograft associated with pretransplantation antibodies directed against donor antigens on endothelium and monocytes. NEJM 300: 1258, 1979c

Paul LC, van Es LA, Fleuren G: Demonstration of transplantation antigens on the endothelium of peritubular capillaries in renal allografts by the immunoperoxidase method. Transplantation 28: 72, 1979d

Paul LC, van Es LA, Baldwin WM: Antigens in human renal allografts. Clin Immunol Immunopathol 19: 206, 1981

Paul LC, Busch GJ, Paradysz JM, Carpenter CB: Definition, genetics, and possible significance of a newly defined endothelial antigen in the rat. Transplantation 36: 533, 1983

Pauli BU, Lee CL: Organ preference of metastasis. The role of organ-specifically modulated endothelial cells. Lab Invest 58: 379, 1988

Pawlowski NA, Abraham EL, Pontier S, Scott WA, Cohn ZA: Human monocyte-endothelial cell interaction in vitro. Proc Natl Acad Sci USA 82: 8208, 1985

Pawlowski NA, Kaplan G, Abraham E, Cohn ZA: The selective binding and transmigration of monocytes through the junctional complexes of human endothelium. J Exp Med 168: 1865, 1988

Pearson JD, Carleton JS, Gordon JL: Metabolism of adenine nucleotides by ectoenzymes of vascular endothelial and smooth-muscle cells in culture. Biochem J 190: 421, 1980

Pederson NC, Morris B: The role of the lymphatic system in the rejection of homografts: a study of lymph from renal transplants. J Exp Med 131: 936, 1970

Penning CA, Cunningham J, French MAH, Harrison G, Rowell NR, Hughes P: Antibody-dependent cellular cytotoxicity of human vascular endothelium in systemic sclerosis. Clin Exp Immunol 58: 548, 1984

Penning CA, French MAH, Rowell NR, Hughes P: Antibody-dependent cellular cytotoxicity of human vascular endothelium in systemic lupus erythematosus. J Clin Lab Immunol 17: 125, 1985

Petty RG, Morgan DML, Pearson JD, Mahler RF: Stimulation of endothelial cell growth by sera from diabetic patients with retinopathy. Lancet 1: 208, 1988

Philips MR, Buyon JP, Winchester R, Weissman G, Abramson SB: Up-regulation of the iC3b receptor (CR3) is neither necessary nor sufficient to promote neutrophil aggregation. J Clin Invest 82: 495, 1988

Phillips DR, Jennings LK, Edwards HH: Identification of membrane proteins mediating the interaction of human platelets. J Cell Biol 86: 77, 1980

Phillips DR, Charo IF, Parise LV, Fitzgerald LA: The platelet membrane glycoprotein IIb-IIIa complex. Blood 71: 831, 1988

Pichyangkul S, Schick D, Schober W, Dixon G, Khan A: Increased expression of adhesive proteins on leukocytes by TNF α. Exp Haematol 16: 588, 1988

Picker LJ, de los Toyos J, Telen MJ, Haynes BF, Butcher EC: Monoclonal antibodies against the CD44 (In (Lu)-related p80), and Pgp-1 antigens in man recognise the Hermes class of lymphocyte homing receptors. J Immunol 142: 2046, 1989

Pierschbacher MD, Ruoslahti E: Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. Nature 309: 30, 1984

Piotrowicz RS, Orchekowski RP, Nugent DJ, Yamada KM, Kunicki TJ: Glycoprotein Ic-IIa functions as an activation-independent fibronectin receptor on human platelets. J Cell Biol 106: 1359, 1988

Pischel KD, Bluestein HG, Woods VL: Very late activation antigens (VLA) are human leukocyte-neuronal crossreactive cell surface antigens. J Exp Med 164: 393, 1986

Pischel KD, Hemler ME, Huang C, Bluestein HG, Woods VL: Use of the monoclonal antibody 12F1 to characterize the differentiation antigen VLA-2. J Immunol 138: 226, 1987

Pischel KD, Bluestein HG, Woods VL: Platelet glycoproteins Ia, Ic, and IIa are physicochemically indistinguishable from the very late activation antigens adhesion-related proteins of lymphocytes and other cell types. J Clin Invest 81: 505, 1988

Pitzalis C, Kingsley G, Haskard D, Parayi G: The preferential accumulation of helper-inducer T lymphocytes in inflammatory lesions: evidence for regulation by selective endothelial and homotypic adhesion. Eur J Immunol 18: 1397, 1988

Plow EF, Srouji AH, Meyer D, Marguerie G, Ginsberg MH: Evidence that three adhesive proteins interact with a common recognition site on activated platelets. J Biol Chem 259: 5388, 1984

Plow EF, McEver RP, Coller BS, Woods VL, Marguerie GA, Ginsberg MH: Related binding mechanisms for fibrinogen, fibronectin, von Willebrand factor, and thrombospondin on thrombin-stimulated human platelets. Blood 66: 724, 1985a

Plow EF, Pierschbacher MD, Ruoslahti E, Marguerie GA, Ginsberg MH: The effects of Arg-Gly-Asp-containing peptides on fibrinogen and von Willebrand factor binding to platelets. Proc Natl Acad Sci USA 82: 8057, 1985b

Plow EF, Loftus JC, Levin EG, Fair DS, Dixon D, Forsyth J, Ginsberg MH: Immunologic relationship between platelet membrane glycoprotein GP IIb/IIIa and cell surface molecules expressed by a variety of cells. Proc Natl Acad Sci USA 83: 6002, 1986

Plunkett ML, Sanders ME, Selvaraj P, Dustin ML, Springer TA: Rosetting of activated human T lymphocytes with autologous erythrocytes. Definition of the receptor and ligand molecules as CD2 and lymphocyte function-associated antigen 3 (LFA-3). J Exp Med 165: 664, 1987

Pober JS, Gimbrone MA: Expression of Ia-like antigens by human vascular endothelial cells is inducible in vitro: demonstration by monoclonal antibody binding and immunoprecipitation. Proc Natl Acad Sci USA 79: 6641, 1982

Pober JS, Gimbrone MA, Cotran RS, Reiss CS, Burakoff SJ, Fiers W, Ault KA: Ia expression by vascular endothelium is inducible by activated T cells and by human γ-interferon. J Exp Med 157: 1339, 1983a

Pober JS, Collins T, Gimbrone MA, Cotran RS, Gitlin JD, Fiers W, Clayberger C, Krensky AM, Burakoff SJ, Reiss CS: Lymphocytes recognise human vascular endothelial and dermal fibroblast Ia antigens induced by recombinant immune interferon. Nature 305: 726, 1983b

Pober JS, Collins T, Gimbrone MA, Libby P, Reiss CS: Inducible expression of class II major histocompatibility complex antigens and the immunogenicity of vascular endothelium. Transplantation 41: 141, 1986a

Pober JS, Bevilacqua MP, Mendrick DL, Lapierre LA, Fiers W, Gimbrone MA: Two distinct monokines, interleukin 1 and tumor necrosis factor, each independently induce biosynthesis and transient expression of the same antigen on the surface of cultured human vascular endothelial cells. J Immunol 136: 1680, 1986b

Pober JS, Gimbrone MA, Lapierre LA, Mendrick DL, Fiers W, Rothlein R, Springer TA: Overlapping patterns of activation of human endothelial cells by interleukin 1, tumor necrosis factor, and immune interferon. J Immunol 137: 1893, 1986c

Pober JS, Lapierre LA, Stolpen AH, Brock TA, Springer TA, Fiers W, Bevilacqua MP, Mendrick DL, Gimbrone MA: Activation of cultured human endothelial cells by recombinant lymphotoxin: comparison with tumor necrosis factor and interleukin 1 species. J Immunol 138: 3319, 1987

Pober JS: Cytokine-mediated activation of vascular endothelium. Physiology and pathology. Am J Pathol 133: 426, 1988

Pohlmann TH, Stanness KA, Beatty PG, Ochs HD, Harlan JM: An endothelial cell surface factor(s) induced in vitro by lipopolysaccharide, interleukin 1, and tumor necrosis factor-α increases neutrophil adherence by a CDw18-dependent mechanism. J Immunol 136: 4548, 1986

Preissner KT, Anders E, Grulich-Henn J, Muller-Berghaus G: Attachment of cultured human endothelial cells is promoted by specific association with S protein (vitronectin) as well as with the ternary S protein-thrombin-antithrombin III complex. Blood 71: 1581, 1988

Prescott SM, Zimmerman GA, McIntyre TM: Human endothelial cells in culture produce platelet-activating factor (1-alkyl-2-acetyl-sn-glycero-3-phosphocholine) when stimulated with thrombin. Proc Natl Acad Sci USA 81: 3534, 1984

Price TH, Beatty PG, Corpuz SR: In vivo inhibition of neutrophil function in the rabbit using monoclonal antibody to CD18. J Immunol 139: 4174, 1987

Prieto J, Beatty PG, Clark EA, Patarroyo M: Molecules mediating adhesion of T and B cells, monocytes, and granulocytes to vascular endothelial cells. Immunol 63: 631, 1988

Pytela R, Pierschbacher MD, Ruoslahti E: Identification and isolation of a 140 kd cell surface glycoprotein with properties expected of a fibronectin receptor. Cell 40: 191, 1985a

Pytela R, Pierschbacher MD, Ruoslahti E: A 125/115-kDa cell surface receptor specific for vitronectin interacts with the arginine-glycine-aspartic acid adhesion sequence derived from fibronectin. Proc Natl Acad Sci USA 82: 5766, 1985b

Pytela R, Pierschbacher MD, Ginsberg MH, Plow EF, Ruoslahti E: Platelet membrane glycoprotein IIb/IIIa: a member of a family of Arg-Gly-Asp-specific adhesion receptors. Science 231: 1559, 1986

Quesenberry PJ, Gimbrone MA: Vascular endothelium as a regulator of granulopoiesis: production of colony-stimulating activity by cultured human endothelial cells. Blood 56: 1060, 1980

Rajavashisth TB, Andalibi A, Territo MC, Berliner JA, Navab M, Fogelman AM, Lusis AJ: Induction of endothelial cell expression of granulocyte and macrophage colony-stimulating factors by modified low-density lipoproteins. Nature 344: 254, 1990

Rapraeger A, Jalkanen M, Bernfield M: Cell surface proteoglycan associates with the cytoskeleton at the basolateral cell surface of mouse mammary epithelial cells. J Cell Biol 103: 2683, 1986

Reichert RA, Gallatin WM, Weissman IL, Butcher EC: Germinal center B cells lack homing receptors necessary for normal lymphocyte recirculation. J Exp Med 157: 813, 1983

Reinherz EL, Morimoto C, Fitzgerald KA, Hassey RE, Daley JF, Schlossman SF: Heterogeneity of human T4+ inducer T cells defined by a monoclonal antibody that delineates two functional subpopulations. J Immunol 128: 463, 1982

Reinholt FP, Hultenby K, Oldberg A, Heinegard D: Osteopontin - a possible anchor of osteoclasts to bone. Proc Natl Acad Sci USA 87: 4473, 1990

Reinsmoen N, Anichini A, Bach FH: Clonal analysis of T lymphocyte response to an isolated class I disparity. Human Immunology 8: 195, 1983

Relman D, Tuomanen E, Falkow S, Golenbock DT, Saukkonen K, Wright SD: Recognition of a bacterial adhesin by an integrin: macrophage CR3 ($\alpha_M\beta_2$, CD11b/CD18) binds filamentous hemagglutinin of Bordetella pertussis. Cell 61: 1375, 1990

Renkonen R, Mattila P, Leszczynski D, Hayry P: Leukotriene B_4 increases the lymphocyte binding to endothelial cells. FEBS Letters 235: 67, 1988

Renkonen R, Mattila P, Turunen JP, Hayry P: Lymphocyte binding to and penetration through vascular endothelium is stimulated by platelet-activating factor. Scand J Immunol 30: 673, 1989a

Renkonen R, Turunen JP, Hayry P: Site of influx of inflammatory white cells into a rejecting rat renal allograft. Transplantation 47: 577, 1989b

Renkonen R, Turunen JP, Rapola J, Hayry P: Characterization of high endothelial-like properties of peritubular capillary endothelium during acute renal allograft rejection. Am J Pathol 137: 643, 1990

Rettig WJ, Dracopoli NC, Goetzger TA, Spengler BA, Biedler JL, Oettgen HF, Old LJ: Somatic cell genetic analysis of human cell surface antigens: chromosomal assignments and regulation of expression in rodent-human hybrid cells. Proc Natl Acad Sci USA 81: 6437, 1984

Rettig WJ, Murty VVVS, Mattes MJ, Chaganti RSK, Old LJ: Extracellular-matrix modulated expression of human cell surface glycoproteins A42 and J143. Intrinsic and extrinsic signals determine antigenic phenotype. J Exp Med 164: 1581, 1986

Rice GE, Gimbrone MA, Bevilacqua MP: Tumor cell-endothelial interactions. Increased adhesion of human melanoma cells to activated vascular endothelium. Am J Pathol 133: 204, 1988

Rice GE, Bevilacqua MP: An inducible endothelial cell surface glycoprotein mediates melanoma adhesion. Science 246: 1303, 1989

Rice GE, Munro JM, Bevilacqua MP: Inducible cell adhesion molecule 110 (INCAM-110) is an endothelial receptor for lymphocytes. A CD11/CD18-independent adhesion mechanism. J Exp Med 171: 1369, 1990

Rock KL, Gamble S, Rothstein L: Presentation of exogenous antigen with class I major histocompatibility complex molecules. Science 249: 918, 1990

Rodriguez M, Pierce ML, Howie EA: Immune response gene products (Ia antigens) on glial and endothelial cells in virus-induced demyelination. J Immunol 138: 3438, 1987

Roos E, Roossien FF: Involvement of leukocyte function-associated antigen-1 (LFA-1) in the invasion of hepatocyte cultures by lymphoma and T-cell hybridoma cells. J Cell Biol 105: 553, 1987

Roossien FF, de Rijk D, Bikker A, Roos E: Involvement of LFA-1 in lymphoma invasion and metastasis demonstrated with LFA-1-deficient mutants. J Cell Biol 108: 1979, 1989

Rose ML, Coles MI, Griffin RJ, Pomerance A, Yacoub MH: Expression of class I and class II major histocompatibility antigens in normal and transplanted human heart. Transplantation 41: 776, 1986

Rosen SD, Singer MS, Yednock TA, Stoolman LM: Involvement of sialic acid on endothelial cells in organ-specific lymphocyte recirculation. Science 228: 1005, 1985

Rosen SD, Chi S, True DD, Singer MS, Yednock TA: Intravenously injected sialidase inactivates attachment sites for lymphocytes on high endothelial venules. J Immunol 142: 1895, 1989

Rosenbaum J, Pottinger BE, Woo P, Black CM, Loizou S, Byron MA, Pearson JD: Measurement and characterisation of circulating anti-endothelial cell IgG in connective tissue diseases. Clin Exp Immunol 72: 450, 1988

Rothlein R, Springer TA: The requirement for lymphocyte function-associated antigen 1 in homotypic leukocyte-adhesion stimulated by phorbol ester. J Exp Med 163: 1132, 1986a

Rothlein R, Dustin ML, Marlin SD, Springer TA: A human intercellular adhesion molecule (ICAM-1) distinct from LFA-1. J Immunol 137: 1270, 1986b

Rothlein R, Czajkowski M, O'Neill MM, Marlin SD, Mainolfi E, Merluzzi VJ: Induction of intercellular adhesion molecule 1 on primary and continuous cell lines by pro-inflammatory cytokines. Regulation by pharmacologic agents and neutralizing antibodies. J Immunol 141: 1665, 1988

Rowe D, Isenberg DA, Beverley PCL: Monoclonal antibodies to human leucocyte antigens in polymyositis and muscular dystrophy. Clin Exp Immunol 54: 327, 1983

Ruoslahti E, Pierschbacher MD: Arg-Gly-Asp: a versatile cell recognition signal. Cell 44: 517, 1986

Ruoslahti E: Proteoglycans in cell regulation. J Biol Chem 264: 13369, 1989

Russ GR, Haddad AP, Tait BD, d'Apice AJF: Polymorphism of the complement receptor for C3bi. J Clin Invest 76: 1965, 1985

Russell DG, Wright SD: Complement receptor type 3 (CR3) binds to an Arg-Gly-Asp-containing region of the major surface glycoprotein, gp63, of Leishmania promastigotes. J Exp Med 168: 279, 1988

Ryan US, Schultz DR, Ryan JW: Fc and C3b receptors on pulmonary endothelial cells: induction by injury. Science 214: 557, 1981

Sacchi A, Falcioni R, Piaggio G, Gianfelice MA, Perrotti N, Kennel SJ: Ligand-induced phosphorylation of a murine tumor surface protein (TSP-180) associated with metastatic phenotype. Cancer Res 49: 2615, 1989

Saegusa Y, Cavender D, Ziff M: Stimulation of mononuclear cell binding to human endothelial cell monolayers by thrombin. J Immunol 141: 4140, 1988

Sage H, Pritzl P, Bornstein P: Characterization of cell matrix associated collagens synthesized by aortic endothelial cells in culture. Biochemistry 20: 436, 1981

Saltini C, Hemler ME, Spurzen J, Crystal R: Expression of the "Very Late Activation Antigen" (VLA-1) complex on lung T-cells characterizes a population of activated nonproliferating T-cells compartmentalized in the normal lung. Am Rev Resp Dis 133: 242, 1986

Sanchez-Madrid R, Krensky AM, Ware CF, Robbins E, Strominger JL, Burakoff SJ, Springer TA: Three distinct antigens associated with human T-lymphocyte-mediated cytolysis: LFA-1, LFA-2, and LFA-3. Proc Natl Acad Sci USA 79: 7489, 1982

Sanchez-Madrid F, Nagy JA, Robbins E, Simon P, Springer TA: A human leukocyte differentiation family with distinct α-subunits and a common β-subunit: the lymphocyte function-associated antigen (LFA-1), the C3bi complement receptor (OKM1/Mac-1), and the p150,95 molecule. J Exp Med 158: 1785, 1983

Sanchez-Madrid F, Toribio ML, Gambon F, De Landazuri MO: Cell surface molecular changes on the activation of human thymocytes. J Immunol 135: 3938, 1985

Sanchez-Madrid F, De Landazuri MO, Morago G, Cebrian M, Acevedo A, Bernabeu C: VLA-3: a novel polypeptide association within the VLA molecular complex: cell distribution and biochemical characterisation. Eur J Immunol 16: 1343, 1986

Sanders ME, Makgoba MW, Sharrow SO, Stephany D, Springer TA, Young HA, Shaw S: Human memory T lymphocytes express increased levels of three cell adhesion molecules (LFA-3, CD2, and LFA-1) and three other molecules (UCHL1, CDw29, and Pgp-1) and have enhanced IFN-γ production. J Immunol 140: 1401, 1988a

Sanders ME, Makgoba MW, Shaw S: Human naive and memory T cells: reinterpretation of helper-inducer and suppressor-inducer subsets. Immunology Today 9: 195, 1988b

Santoro SA: Identification of a 160,000 dalton platelet membrane protein that mediates the initial divalent cation-dependent adhesion of platelets to collagen. Cell 46: 913, 1986

Santoro SA, Lawing WJ: Competition for related but nonidentical binding sites on the glycoprotein IIb-IIIa complex by peptides derived from platelet adhesive proteins. Cell 48: 867, 1987

Saunders S, Bernfield M: Cell surface proteoglycan binds mouse mammary epithelial cells to fibronectin and behaves as a receptor for interstitial matrix. J Cell Biol 106: 423, 1988

Savill J, Dransfield I, Hogg N, Haslett C: Vitronectin receptor-mediated phagocytosis of cells undergoing apoptosis. Nature 343: 170, 1990

Schainberg H, Borish L, King M, Rocklin RE, Rosenwasser LJ: Leukocyte inhibitory factor stimulates neutrophil-endothelial cell adhesion. J Immunol 141: 3055, 1988

Schaper RJ, van Dinther-Janssen ACHM, Polak L: Specific accumulation of hapten-reactive T cells in contact sensitivity reaction sites. J Immunol 134: 1333, 1985

Schindler M, Meiners S, Cheresh DA: RGD-dependent linkage between plant cell wall and plasma membrane: consequences for growth. J Cell Biol 108: 1955, 1989

Schleimer RP, Rutledge BK: Cultured human vascular endothelial cells acquire adhesiveness for neutrophils after stimulation with interleukin 1, endotoxin, and tumor-promoting phorbol diesters. J Immunol 136: 649, 1986

Schoefl GI: The migration of lymphocytes across the vascular endothelium in lymphoid tissue. A reexamination. J Exp Med 136: 568, 1972

Schook LB, Wood N, Mohanakumar T: Identification of human vascular endothelial cell/monocyte antigenic system using monoclonal antibodies. Transplantation 44: 412, 1987

Schreiber AB, Kenney J, Kowalski WJ, Friesel R, Mehlman T, Maciag T: Interaction of endothelial cell growth factor with heparin: characterisation by receptor and antibody recognition. Proc Natl Acad Sci USA 82: 6138, 1985a

Schreiber AB, Kenney J, Kowalski J, Thomas KA, Gimenez-Galtego G, Rios-Candelore M, Di Salvo J, Barritault D, Courty J, Courtois Y, Moenner M, Loret C, Burgess WH, Hehlman T, Friesel R, Johnson W, Maciag T: A unique family of endothelial cell polypeptide mitogens: the antigenic and receptor cross-reactivity of bovine endothelial cell growth factor, brain-derived acidic fibroblast growth factor, and eye-derived growth factor-V. J Cell Biol 101: 1623, 1985

Schulman M, Wilde C, Kohler G: A better cell line for making hybridomas secreting specific antibodies. Nature 276: 269, 1978

Schwarting R, Stein H, Wang CY: The monoclonal antibody αS-HCL 1 (αLeu-14) and αS-HCL 3 (αLeu-M5) allow the diagnosis of Hairy cell leukaemia. Blood 65: 974, 1985

Schwartz BR, Ochs HD, Beatty PG, Harlan JM: A monoclonal antibody-defined membrane antigen complex is required for neutrophil-neutrophil aggregation. Blood 65: 1553, 1985

Schwartz BR, Wayner EA, Carlos TM, Ochs HD, Harlan JM: Identification of surface proteins mediating adherence of CD11/CD18-deficient lymphoblastoid cells to cultured human endothelium. J Clin Invest 85: 2019, 1990

Schwarz RE, Hiserodt JC: The expression and functional involvement of laminin-like molecules in non-MHC restricted cytotoxicity by human Leu-19$^+$/CD3$^-$ natural killer lymphocytes. J Immunol 141: 3318, 1988

Segal GM, McCall E, Stueve T, Bagby GC: Interleukin 1 stimulates endothelial cells to release multilineage human colony-stimulating activity. J Immunol 138: 1772, 1987

Selvaraj P, Plunkett ML, Dustin M, Sanders ME, Shaw S, Springer TA: The T lymphocyte glycoprotein CD2 binds the cell surface ligand LFA-3. Nature 326: 400, 1987a

Selvaraj P, Dustin ML, Silber R, Low MG, Springer TA: Deficiency of lymphocyte function-associated antigen 3 (LFA-3) in paroxysmal nocturnal haemoglobinuria. Functional correlates and evidence for a phosphatidyl membrane anchor. J Exp Med 166: 1011, 1987b

van Seventer GA, Shimizu Y, Horgan KJ, Shaw S: The LFA-1 ligand ICAM-1 provides an important costimulatory signal for T cell receptor-mediated activation of resting T cells. J Immunol 144: 4579, 1990

Shanahan WR, Weston DM, Korn JH: Endothelial cells enhance the human pokeweed mitogen lymphocyte response. J Leukocyte Biology 37: 305, 1985

Sharon N, Lis H: Lectins as cell recognition molecules. Science 246: 227, 1989

Shattil SJ, Hoxie JA, Cunningham M, Brass LF: Changes in the platelet membrane glycoprotein IIb-IIIa complex during platelet activation. J Biol Chem 260: 11107, 1985

Shaw S, Luce GEG, Quinones R, Gress RE, Springer TA, Sanders ME: Two antigen-independent adhesion pathways used by human cytotoxic T-cell clones. Nature 323: 262, 1986

Shaw S, Ginther Luce GE: The lymphocyte function-associated antigen (LFA-1) and CD2/LFA-3 pathways of antigen-independent T cell adhesion. J Immunol 139: 1037, 1987

Sheppard D, Rozzo C, Starr L, Quaranta V, Erle DJ, Pytela R: Complete amino acid sequence of a novel integrin β subunit (β_6) identified in epithelial cells using the polymerase chain reaction. J Biol Chem 265: 11502, 1990

Shimizu Y, van Seventer GA, Siraganian R, Wahl L, Shaw S: Dual role of the CD44 molecule in T cell adhesion and activation. J Immunol 143: 2457, 1989

Shimizu Y, van Seventer GA, Horgan KJ, Shaw S: Regulated expression and binding of three VLA (β_1) integrin receptors on T cells. Nature 345: 250, 1990a

Shimizu Y, van Seventer GA, Horgan KJ, Shaw S: Costimulation of proliferative responses of resting $CD4^+$ T cells by the interaction of VLA-4 and VLA-5 with fibronectin or VLA-6 with laminin. J Immunol 145: 59, 1990b

Shiohara T, Moriya N, Gotoh C, Saizawa K, Nagashima M: Locally administered monoclonal antibodies to lymphocyte function-associated antigen 1 and to L3T4 prevent cutaneous graft-vs-host disease. J Immunol 141: 2261, 1988

Siegelman M, Bond MW, Gallatin M, St. John T, Smith HT, Fried VA, Weissman IL: Cell surface molecule associated with lymphocyte homing is a ubiquinated branched-chain glycoprotein. Science 231: 823, 1986

Siegelman MH, Weissman IL: Human homologue of mouse lymph node homing receptor: evolutionary conservation at tandem cell interaction domains. Proc Natl Acad Sci USA 86: 5562, 1989

Simmons D, Makgoba MW, Seed B: ICAM-1, an adhesion ligand of LFA-1, is homologous to the neural cell adhesion molecule NCAM. Nature 331: 624, 1988

Simpson PJ, Todd RF, Fontone JC, Mickelson JK, Griffin JD, Lucchesi BR: Reduction of experimental canine myocardial reperfusion injury by a monoclonal antibody (anti-Mol, anti-CD11b) that inhibits leukocyte adhesion. J Clin Invest 81: 624, 1988

Sironi M, Breviario F, Proserpio P, Biondi A, Vecchi A, Van Damme J, Dejana E, Mantovani A: IL-1 stimulates IL-6 production in endothelial cells. J Immunol 142: 549, 1989

Siu G, Hedrick SM, Brian AA: Isolation of the murine intercellular adhesion molecule 1 (ICAM-1) gene. ICAM-1 enhances antigen-specific T cell activation. J Immunol 143: 3813, 1989

Skorkiewicz MJ, Colvin RB, Schreeberger EE, Russell PS: Widespread and selective induction of major histocompatibility complex-determined antigens in vivo by γ-interferon. J Exp Med 162: 1645, 1985

Smith CW, Rothlein R, Hughes BJ, Marisealco MM, Rudloff HE, Schmalstieg FC, Anderson DC: Recognition of an endothelial determinant for CD18-dependent human neutrophil adherence and transendothelial migration. J Clin Invest 82: 1746, 1988

Smith CW, Marlin SD, Rothlein R, Toman C, Anderson DC: Cooperative interactions of LFA-1 and Mac-1 with intercellular adhesion molecule-1 in facilitating adherence and transendothelial migration of human neutrophils in vitro. J Clin Invest 83: 2008, 1989

Smith JW, Vestal DJ, Irwin SV, Burke TA, Cheresh DA: Purification and functional characterization of integrin $\alpha_V\beta_5$. An adhesion receptor for vitronectin. J Biol Chem 265: 11008, 1990a

Smith JW, Ruggeri ZM, Kunicki TJ, Cheresh DA: Interaction of integrins $\alpha_V\beta_3$ and glycoprotein IIb-IIIa with fibrinogen. Differential peptide recognition accounts for distinct binding sites. J Biol Chem 265: 12267, 1990b

Sobel RA, Blanchette BW, Bhan AK, Colvin RB: The immunopathology of experimental allergic encephalomyelitis: II: endothelial cell Ia increases prior to inflammatory cell infiltration. J Immunol 132: 2402, 1984

Somers SD, Whisnant CC, Adams DO: Quantification of the strength of cell-cell adhesion: the capture of tumor cells by activated murine macrophages proceeds through two distinct stages. J Immunol 136: 1490, 1986

Sonnenberg A, Daams H, van der Valk MA, Hilkens J, Hilgers J: Development of mouse mammary gland: identification of stages in differentiation of luminal and myoepithelial cells using monoclonal antibodies and polyvalent antiserum against keratin. J Histochem Cytochem 34: 1037, 1986

Sonnenberg A, Janssen H, Hogervorst F, Calafat J, Hilgers J: A complex of platelet glycoproteins Ic and IIa identified by a rat monoclonal antibody. J Biol Chem 262: 10376, 1987

Sonnenberg A, Hogervorst F, Osterop A, Veltman FEM: Identification and characterisation of a novel antigen complex on mouse mammary tumor cells using a monoclonal antibody against platelet glycoprotein Ic. J Biol Chem 263: 14030, 1988a

Sonnenberg A, Modderman PW, Hogervorst F: Laminin receptor on platelets is the integrin VLA-6. Nature 336: 487, 1988b

Sonnenberg A, Linders CJT, Modderman PW, Damsky CH, Aumailley M, Timpl R: Integrin recognition of different cell-binding fragments of laminin (P1, E3, E8) and evidence that $\alpha_6\beta_1$ but not $\alpha_6\beta_4$ functions as a major receptor for fragment E8. J Cell Biol 110: 2145, 1990

Spits H, Ijsell H, Terhorst C, de Vries JE: Establishment of human T lymphocyte clones highly cytotoxic for an EBV-transformed B cell line in serum-free medium: isolation of clones that differ in phenotype and specificity. J Immunol 128: 95, 1982

Spits H, van Schooten W, Keizer H, van Seventer G, van de Rijn M, Terhorst C, de Vries JE: Alloantigen recognition is preceded by nonspecific adhesion of cytotoxic T cells and target cells. Science 232: 403, 1986

Sporn SA, Eierman DF, Johnson CE, Morris J, Martin G, Ladner M, Haskill S: Monocyte adherence results in selective induction of novel genes sharing homology with mediators of inflammation and tissue repair. J Immunol 144: 4434, 1990

Springer TA, Galfre G, Secher DS, Milstein C: Mac-1: a macrophage differentiation antigen defined by monoclonal antibody. Eur J Immunol 9: 301, 1979

Springer TA, Miller LJ, Anderson DC: p150,95, the third member of the Mac-1, LFA-1 human leukocyte adhesion glycoprotein family. J Immunol 136: 240, 1986

Springer TA, Dustin ML, Kishimoto TK, Marlin SD: The lymphocyte function-associated LFA-1, CD2, and LFA-3 molecules: cell adhesion receptors of the immune system. Ann Rev Immunol 5: 223, 1987

Springer WR, Cooper DNW, Barondes SH: Discoidin 1 is implicated in cell-substratum attachment and ordered cell migration of Dictyostelium discoideum and resembles fibronectin. Cell 39: 557, 1984

Staatz WD, Rajpara SM, Wayner EA, Carter WG, Santoro SA: The membrane glycoprotein Ia-IIa (VLA-2) complex mediates the Mg^{++}-dependent adhesion of platelets to collagen. J Cell Biol 108: 1917, 1989

Staatz WD, Walsh JJ, Pexton T, Santoro SA: The $\alpha_2\beta_1$ integrin cell surface collagen receptor binds to the α1(I)-CB3 peptide of collagen. J Biol Chem 265: 4778, 1990

Stamenkovic I, Amiot M, Pesando JM, Seed B: A lymphocyte molecule implicated in lymph node homing is a member of the cartilage link protein family. Cell 56: 1057, 1989

Stampfer HB, Woodruff JJ: Lymphocyte homing into lymph nodes: in vitro demonstration of the selective affinity of recirculating lymphocytes for high-endothelial venules. J Exp Med 144: 828, 1976

Staunton DE, Marlin SD, Stratowa C, Dustin ML, Springer TA: Primary structure of ICAM-1 demonstrates interaction between members of the immunoglobulin and integrin supergene families. Cell 52: 925, 1988

Staunton DE, Merluzzi VJ, Rothlein R, Barton R, Marlin SD, Springer TA: A cell adhesion molecule, ICAM-1, is the major surface receptor for rhinoviruses. Cell 56: 849, 1989a

Staunton DE, Dustin ML, Springer TA: Functional cloning of ICAM-2, a cell adhesion ligand for LFA-1 homologous to ICAM-1. Nature 339: 61, 1989b

Staunton DE, Dustin ML, Erickson HP, Springer TA: The arrangement of the immunoglobulin-like domains of ICAM-1 and the binding sites for LFA-1 and rhinovirus. Cell 61: 243, 1990

Stegall MD, Tezuka K, Oluwole SF, Engelsted K, Jing MX, Andrew J, Hardy MA: Interstitial class II-positive cell depletion by donor pretreatment with gamma irradiation. Evidence of differential immunogenicity between vascularised cardiac allografts and islets. Transplantation 49: 246, 1990

Stern DM, Drillings M, Nossel HL, Hurlet-Jensen A, LeGamma KS, Owen J: Binding of factors IX and IXa to cultured vascular endothelial cells. Proc Natl Acad Sci USA 80: 4119, 1983

Stern DM, Nawroth P, Marcum J, Handley D, Kisiel W, Rosenberg R, Stern K: Interaction of antithrombin III with bovine aortic segments. Role of heparin in binding and enhanced anticoagulant activity. J Clin Invest 75: 272, 1985a

Stern DM, Bank I, Nawroth PP, Cassimeris J, Kisiel W, Fenton JW, Dinarello C, Chess L, Jaffe EA: Self-regulation of procoagulant events on the endothelial cell surface. J Exp Med 162: 1223, 1985b

Stevens SK, Weissman IL, Butcher EC: Differences in the migration of B and T lymphocytes: organ-selective localization in vivo and the role of lymphocyte-endothelial cell recognition. J Immunol 128: 844, 1982

St John T, Meyer J, Idzerda R, Gallatin WM: Expression of CD44 confers a new adhesive phenotype on transfected cells. Cell 60: 45, 1990

Stolpen AH, Guinan EC, Fiers W, Pober JS: Recombinant tumor necrosis factor and immune interferon act singly and in combination to reorganize human vascular endothelial cell monolayers. Am J Pathol 123: 16, 1986

Stoolman LM, Rosen SD: Possible role for cell-surface carbohydrate-binding molecules in lymphocyte recirculation. J Cell Biol 96: 722, 1983

Stoolman LM, Yednock TA, Rosen SD: Homing receptors on human and rodent lymphocytes - evidence for a conserved carbohydrate-binding specificity. Blood 70: 1842, 1987

Stoolman LM: Adhesion molecules controlling lymphocyte migration. Cell 56: 907, 1989a

Stoolman LM, Ebling H: Adhesion molecules of cultured hematopoietic malignancies. A calcium-dependent lectin is the principal mediator of binding to the high endothelial venule of lymph nodes. J Clin Invest 84: 1196, 1989b

Strassman G, Springer TA, Somers SD, Adams DO: Mechanisms of tumor cell capture by activated macrophages: evidence for involvement of lymphocyte function-associated (LFA) -1 antigen. J Immunol 136: 4328, 1986

Straus AH, Carter WG, Wayner EA, Hakemori SI: Mechanism of fibronectin-mediated cell migration: dependence or independence of cell migration susceptibility on RGDS-directed receptor (integrin). Exp Cell Res 183: 126, 1989

Streeter PR, Berg EL, Rouse BTN, Bargatze RF, Butcher EC: A tissue-specific endothelial cell molecule involved in lymphocyte homing. Nature 331: 41, 1988a

Streeter PR, Rouse BTN, Butcher EC: Immunohistologic and functional characterization of a vascular addressin involved in lymphocyte homing into peripheral lymph nodes. J Cell Biol 107: 1853, 1988b

Streuli CH, Bissell MJ: Expression of extracellular matrix components is regulated by substratum. J Cell Biol 110: 1405, 1990

Strieter RM, Kunkel SL, Showell HJ, Remick DG, Pham SH, Ward PA, Marks RM: Endothelial cell gene expression of a neutrophil chemotactic factor by TNF-α, LPS, and IL-1β. Science 243: 1467, 1989

de Strooper B, van der Schueren B, Jaspers M, Saison M, Spaepen M, van Leuven F, van den Berghe H, Cassiman JJ: Distribution of the β_1 subgroup of the integrins in human cells and tissues. J Histochem Cytochem 37: 299, 1989

Suzuki S, Oldberg A, Hayman EG, Pierschbacher MD, Ruoslahti E: Complete amino acid sequence of human vitronectin deduced from cDNA. Similarity of cell attachment sites in vitronectin and fibronectin. EMBO J 4: 2519, 1985

Suzuki S, Argraves WS, Pytela R, Arai H, Krusius T, Pierschbacher MD, Ruoslahti E: cDNA and amino acid sequences of the cell adhesion protein receptor recognizing vitronectin reveal a transmembrane domain and homologies with other adhesion protein receptors. Proc Natl Acad Sci USA 83: 8614, 1986

Suzuki S, Naitoh Y: Amino acid sequence of a novel integrin β_4 subunit and primary expression of the mRNA in epithelial cells. EMBO J 9: 757, 1990

Sviland L, Pearson ADJ, Eastham EJ, Green MA, Hamilton PJ, Proctor SJ, Malcolm AJ: Class II antigen expression by keratinocytes and enterocytes - an early feature of graft-versus-host disease. Transplantation 46: 402, 1988

Symington BE, Symington FW, Rohrschneider LR: Phorbol ester induces increased expression, altered glycosylation, and reduced adhesion of K562 erythroleukaemia cell fibronectin receptors. J Biol Chem 264: 13258, 1989

Takada Y, Huang C, Hemler ME: Fibronectin receptor structures in the VLA family of heterodimers. Nature 326: 607, 1987a

Takada Y, Strominger JL, Hemler ME: The very late antigen family of heterodimers is part of a superfamily of molecules involved in adhesion and embryogenesis. Proc Natl Acad Sci USA 84: 3239, 1987b

Takada Y, Wayner EA, Carter WG, Hemler ME: Extracellular matrix receptors, ECMR II and ECMR I, for collagen and fibronectin correspond to VLA-2 and VLA-3 in the VLA family of heterodimers. J Cell Biochem 37: 385, 1988

Takada Y, Elices MJ, Crouse C, Hemler ME: The primary structure of the α_4 subunit of VLA-4: homology to other integrins and a possible cell-cell adhesion function. EMBO J 8: 1361, 1989a

Takada Y, Hemler ME: The primary structure of the VLA-2/collagen receptor α_2 subunit (platelet GP Ia): homology to other integrins and the presence of a possible collagen-binding domain. J Cell Biol 109: 397, 1989b

Takai Y, Reed ML, Burakoff SJ, Herrman SH: Direct evidence for a receptor-ligand interaction between the T-cell surface antigen CD2 and lymphocyte-function-associated antigen 3. Proc Natl Acad Sci USA 84: 6864, 1987

Takehara K, LeRoy EC, Grotendorst GR: TGF-β inhibition of endothelial cell proliferation: alteration of EGF binding and EGF-induced growth-regulatory (competence) gene expression. Cell 49: 415, 1987

Takei F: Inhibition of mixed lymphocyte response by a rat monoclonal antibody to a novel murine lymphocyte activation antigen (MALA 2). J Immunol 134: 1403, 1985

Talamas-Rohana P, Wright SD, Lennartz MR, Russell DG: Lipophosphoglycan from Leishmania mexicana promastigotes binds to members of the CR3, p150,95 and LFA-1 family of leukocyte integrins. J Immunol 144: 4817, 1990

Tamkun JW, DeSimone DW, Fonde D, Patel RS, Buck C, Horwitz AF, Hynes RO: Structure of integrin, a glycoprotein involved in the transmembrane linkage between fibronectin and actin. Cell 46: 271, 1986

Tamura RN, Rozzo C, Starr L, Chambers J, Reichardt LF, Cooper HM, Quaranta V: Epithelial forms of $\alpha_6\beta_4$: complete primary structure of α_6 and variant forms of β_4. J Cell Biol 111: 1593, 1990

Tedder TF, Isaacs CM, Ernst TJ, Demetri GD, Adler DA, Disteche CM: Isolation and chromosomal localization of cDNAs encoding a novel human lymphocyte cell surface molecule LAM-1. Homology with the mouse lymphocyte homing receptor and other human adhesion proteins. J Exp Med 170: 123, 1989

Tedder TF, Penta AC, Levine HB, Freedman AS: Expression of the human leukocyte adhesion molecule, LAM1. Identity with the TQ1 and Leu-8 differentiation antigens. J Immunol 144: 532, 1990

Te Velde AA, Keizer GD, Figdor CG: Differential function of LFA-1 family molecules (CD11 and CD18) in adhesion of human monocytes to melanoma and endothelial cells. Immunology 61: 261, 1987

Thiagarajan P, Shapiro SS, Levine E, De Marco L, Yalein A: A monoclonal antibody to human platelet glycoprotein IIIa detects a related protein in cultured human endothelial cells. J Clin Invest 75: 896, 1985

Thiagarajan P, Kelly KL: Exposure of binding sites for vitronectin on platelets following stimulation. J Biol Chem 263: 3035, 1988

Thomas PD, Hampson FW, Casale JM, Hunninghake GW: Neutrophil adherence to human endothelial cells. J Lab Clin Med 111: 286, 1988

Thompson JS, Overlin V, Severson CD, Parsons TJ, Herbick J, Straus RG, Burns CP, Claas FHJ: Demonstration of granulocyte, monocyte, and endothelial cell antigens by double fluorochromatic microcytotoxicity testing. Trans Proc 12: 26, 1980

Thorens B, Mermod JJ, Vassalli P: Phagocytosis and inflammatory stimuli induce GM-CSF mRNA in macrophages through post-transcriptional regulation. Cell 48: 671, 1987

Thornhill MH, Kyan-Aung U, Haskard DO: IL-4 increases human endothelial cell adhesiveness for T cells but not for neutrophils. J Immunol 144: 3060, 1990

Thornton SC, Mueller SN, Levine EM: Human endothelial cells: use of heparin in cloning and long-term serial cultivation. Science 222: 623, 1983

Tilney NL, Notis-McConarty J, Strom TB: Specificity of cellular migration into cardiac allografts in rats. Transplantation 26: 181, 1978

Timonen T, Patarroyo M, Gahmberg CG: CD11a-c/CD18 and GP84 (LB-2) adhesion molecules on human large granular lymphocytes and their participation in natural killing. J Immunol 141: 1041, 1988

Timpl R: Structure and biological activity of basement membrane proteins. Eur J Biochem 180: 487, 1989

Todd RF, Nadler LM, Schlossman SF: Antigens on human monocytes identified by monoclonal antibodies. J Immunol 126: 1435, 1981

Todd RF, van Agthoven A, Schlossman SF, Terhorst C: Structural analysis of differentiation antigens Mo1 and Mo2 on human monocytes. Hybridoma 1: 329, 1982

Todd RF, Arnaout MA, Rosin RE, Crowley CA, Peters WA, Babior BM: Subcellular localization of the large subunit of Mo1 (Mo1α; formerly gp110), a surface glycoprotein associated with neutrophil adhesion. J Clin Invest 74: 1280, 1984

Toma H, Tanabe K, Oba S, Horita S, Takahashi K, Teraoka S, Ota K: Expression of class II antigens in human renal allografts: comparison of acute rejection, chronic rejection, and cyclosporine nephrotoxicity. Trans Proc 19: 3719, 1987

Tomaselli KJ, Damsky CH, Reichardt LF: Purification and characterization of mammalian integrins expressed by a rat neuronal cell line (PC12): evidence that they function as α/β heterodimeric receptors for laminin and type IV collagen. J Cell Biol 107: 1241, 1988

Tonneson MG, Smedly LA, Henson PM: Neutrophil-endothelial cell interactions. Modulation of neutrophil adhesiveness induced by complement fragments C5a and C5a des arg and formyl-methionyl-leucyl-phenylalanine in vitro. J Clin Invest 74: 1581, 1984

Tonneson MG, Anderson DC, Springer TA, Knedler A, Avdi N, Henson PM: Adherence of neutrophils to cultured human microvascular endothelial cells. Stimulation by chemotactic peptides and lipid mediators and dependence upon the Mac-1, LFA-1, p150,95 glycoprotein family. J Clin Invest 83: 637, 1989

Torok-Storb B, Nepom GT, Nepom BS, Hansen JA: HLA-DR antigens on lymphoid cells differ from those on myeloid cells. Nature 305: 541, 1983

Tosato G, Pike SE: Interferon-β_2/interleukin 6 is a co-stimulant for human T lymphocytes. J Immunol 141: 1556, 1988

Tosato G, Miller J, Marti G, Pike SE: Accessory function of interleukin-1 and interleukin-6: preferential costimulation of T4 positive lymphocytes. Blood 75: 922, 1990

Totterman TH, Hanas E, Bergstrom R, Larsson E, Tufveson G: Immunologic diagnosis of kidney rejection using FACS analysis of graft-infiltrating functional and activated T and NK cell subsets. Transplantation 47: 817, 1989

Townshend A, McMichael A: Those images that yet fresh images beget. Nature 329: 482, 1987

Trowsdale J: Molecular genetics of the MHC. Immunology (supp 1): 21, 1988

Tuomanen EI, Saukkonen K, Sande S, Cioffe C, Wright SD: Reduction of inflammation, tissue damage, and mortality in bacterial meningitis in rabbits treated with monoclonal antibodies against adhesion-promoting receptors of leukocytes. J Exp Med 170: 959, 1989

Unanue ER, Allen PM: The basis for the immunoregulatory role of macrophages and other accessory cells. Science 236: 551, 1987

Umetsu DT, Katzen D, Jabora HH, Geha RS: Antigen presentation by human dermal fibroblasts: activation of resting T lymphocytes. J Immunol 136: 440, 1986

Vedder NB, Harlan JM: Increased surface expression of CD11b/CD18 (Mac-1) is not required for stimulated neutrophil adherence to cultured endothelium. J Clin Invest 81: 676, 1988a

Vedder NB, Winn RK, Rice CL, Chi EY, Arfors KE, Harlan JM: A monoclonal antibody to the adherence-promoting leukocyte glycoprotein, CD18, reduces organ injury and improves survival from haemorrhagic shock and resuscitation in rabbits. J Clin Invest 81: 939, 1988b

Vedder NB, Winn RK, Rice CL, Chi EY, Arfors KE, Harlan JM: Inhibition of leukocyte adherence by anti-CD18 monoclonal antibody attenuates reperfusion injury in the rabbit ear. Proc Natl Acad Sci USA 87: 2643, 1990

Vismara A, Meroni PL, Tincani A, Harris EN, Barcellini W, Brucato A, Khamashta M, Hughes GRV, Zanussi C, Balestrieri G: Relationship between anti-cardiolipin and anti-endothelial cell antibodies in systemic lupus erythematosus. Clin Exp Immunol 74: 247, 1988

Vogel BE, Tarone G, Giancotti FG, Gailit J, Ruoslahti E: A novel fibronectin receptor with an unexpected subunit composition ($\alpha_V\beta_1$). J Biol Chem 265: 5934, 1990

Volger LW, Tuck DT, Springer TA, Haynes BF, Singer KH: Thymocyte binding to human thymic epithelial cells is inhibited by monoclonal antibodies to CD2 and LFA-3 antigens. J Immunol 138: 358, 1987

de Waal RMW, Bogman MJJ, Maass CN, Cornelissen LMH, Tax WJM, Koene RAP: Variable expression of Ia antigens on the vascular endothelium of mouse skin allografts. Nature 303: 426, 1983

Wacholtz MC, Patel SS, Lipsky PE: Leukocyte function-associated antigen 1 is an activation molecule for human T cells. J Exp Med 170: 431, 1989

Wadgymar A, Ritchie S, Cattran DC, Fenton S, Halloran PF: Patterns of HLA antigen expression in human kidney disease. Trans Proc 19: 3410, 1987

Wagner CR, Vetto RM, Burger DR: The mechanism of antigen presentation by endothelial cells. Immunobiology 168: 453, 1984

Wagner CR, Vetto RM, Burger DR: Subcultured human endothelial cells can function independently as fully competent antigen-presenting cells. Human Immunology 13: 33, 1985a

Wagner CR, Vetto RM, Burger DR: Expression of I-region-associated antigen (Ia) and interleukin 1 by subcultured human endothelial cells. Cell Immunol 93: 91, 1985b

Wagner DD, Olmsted JB, Mardler VJ: Immunolocalisation of von Willebrand protein in Weibel-Palade bodies of human endothelial cells. J Cell Biol 95: 355, 1982

Wallis WJ, Beatty PG, Ochs HD, Harlan JM: Human monocyte adherence to cultured vascular endothelium: monoclonal antibody-defined mechanisms. J Immunol 135: 2323, 1985

Wallis WJ, Hickstein DD, Schwartz BR, June CH, Ochs HD, Beatty PG, Klebanoff SJ, Harlan JM: Monoclonal antibody-defined functional epitopes on the adhesion-promoting glycoprotein complex (CDw18) of human neutrophils. Blood 67: 1007, 1986

Wallner BP, Frey AZ, Tizard R, Mattaliano RJ, Hession C, Sanders ME, Dustin ML, Springer TA: Primary structure of lymphocyte function-associated antigen 3 (LFA-3): the ligand of the T lymphocyte CD2 glycoprotein. J Exp Med 166: 923, 1987

Walther BT, Ohman R, Roseman S: A quantitative assay for intercellular adhesion. Proc Natl Acad Sci USA 70: 1569, 1973

Wang D, Liebowitz D, Wang F, Gregory C, Rickinson A, Larson R, Springer T, Kieff E: Epstein-Barr virus latent infection membrane protein alters the human B-lymphocyte phenotype: deletion of the amino terminus abolishes activity. J Virology 62: 4173, 1988

Wantzin GL, Ralfkiaer E, Lisby S, Rothlein R: The role of intercellular adhesion molecules in inflammatory skin reactions. Br J Dermatol 119: 141, 1988

Wardlaw AJ, Hibbs ML, Stacker SA, Springer TA: Distinct mutations in two patients with leukocyte adhesion deficiency and their functional correlates. J Exp Med 172: 335, 1990

Warner SJC, Auger KR, Libby P: Interleukin 1 induces interleukin 1. II. Recombinant human interleukin 1 induces interleukin 1 production by adult human vascular endothelial cells. J Immunol 139: 1911, 1987

Watson ML, Kingsmore SF, Johnston GI, Siegelman MH, LeBeau MM, Lemons RS, Bora NS, Howard TA, Weissman IL, McEver RP, Seldin MF: Genomic organization of the selectin family of leukocyte adhesion molecules on human and mouse chromosome 1. J Exp Med 172: 263, 1990

Wawryk SO, Novotny JR, Wicks IP, Wilkinson D, Maher D, Salvaris E, Welch K, Fecondo J, Boyd AW: The role of the LFA-1/ICAM-1 interaction in human leukocyte homing and adhesion. Immunol Rev 108: 135, 1989

Wayner EA, Carter WG: Identification of multiple cell adhesion receptors for collagen and fibronectin in human fibrosarcoma cells possessing unique α and common β subunits. J Cell Biol 105: 1873, 1987

Wayner EA, Carter WG, Piotrowicz RS, Kunicki TJ: The function of multiple extracellular matrix receptors in mediating cell adhesion to extracellular matrix: preparation of monoclonal antibodies to the fibronectin receptor that specifically inhibit cell adhesion to fibronectin and react with platelet glycoproteins Ic-IIa. J Cell Biol 107: 1881, 1988

Wayner EA, Garcia-Pardo A, Humphries MJ, McDonald JA, Carter WG: Identification and characterization of the T lymphocyte adhesion receptor for an alternative cell attachment domain (CS-1) in plasma fibronectin. J Cell Biol 109: 1321, 1989

Webb DSA, Shimizu Y, van Seventer GA, Shaw S, Gerrard TL: LFA-3, CD44, and CD45: physiologic triggers of human monocyte TNF and IL-1 release. Science 249: 1295, 1990

Wee SL, Chen LK, Strassman G, Bach FH: Helper cell-independent cytotoxic clones in man. J Exp Med 156: 1854, 1982

Weetman AP, Rees AJ: Synergistic effects of recombinant tumor necrosis factor and interferon-gamma on rat thyroid cell growth and Ia antigen expression. Immunology 63: 285, 1988

Weetman AP, Cohen S, Makgoba MW, Borysiewicz LK: Expression of an intercellular adhesion molecule, ICAM-1, by human thyroid cells. J Endocrinol 122: 185, 1989

Wegner CD, Gundel RH, Reilly P, Haynes N, Letts LG, Rothlein R: Intercellular adhesion molecule-1 (ICAM-1) in the pathogenesis of asthma. Science 247: 456, 1990

Weibel ER, Palade GE: New cytoplasmic components in arterial endothelia. J Cell Biol 23: 101, 1964

Weksler BB, Marcus AJ, Jaffe EA: Synthesis of prostaglandin I_2 (prostacyclin) by cultured human and bovine endothelial cells. Proc Natl Acad Sci USA 74: 3922, 1977

Weksler BB, Ley CW, Jaffe EA: Stimulation of endothelial cell prostacyclin production by thrombin, trypsin, and the ionophore A 23187. J Clin Invest 62: 923, 1978

Wellicome SM, Thornhill MH, Pitzalis C, Thomas DS, Lanchbury JSS, Panayi GS, Haskard DO: A monoclonal antibody that detects a novel antigen on endothelial cells that is induced by tumor necrosis factor, IL-1, or lipopolysaccharide. J Immunol 144: 2558, 1990

Werb Z, Tremble PM, Behrendtsen O, Crowley E, Damsky CH: Signal transduction through the fibronectin receptor induces collagenase and stromelysin gene expression. J Cell Biol 109: 877, 1989

Wheeler ME, Luscinskas FW, Bevilacqua MP, Gimbrone MA: Cultured human endothelial cells stimulated with cytokines or endotoxin produce an inhibitor of leukocyte adhesion. J Clin Invest 82: 1211, 1988

White JM, Littman DR: Viral receptors of the immunoglobulin superfamily. Cell 56: 725, 1989

Wilcox M, Leptin M: Tissue-specific modulation of a set of related cell surface antigens in Drosophila. Nature 316: 351, 1985

Williams AF, Barclay AN: The immunoglobulin superfamily - domains for cell surface recognition. Ann Rev Immunol 6: 381, 1988

Wood NL, Schook LB, Studer EJ, Mohanakumar T: Biochemical characterisation of human vascular endothelial cell-monocyte antigens defined by monoclonal antibodies. Transplantation 45: 787, 1988

Woodruff JJ, Clarke LM, Chin YH: Specific cell-adhesion mechanisms determining migration pathways of recirculating lymphocytes. Ann Rev Immunol 5: 201, 1987

Woods VL, Pischel KD, Avery ED, Bluestein HG: Antigenic polymorphism of human very late activation protein-2 (platelet glycoprotein Ia-IIa). Platelet alloantigen Hc^a. J Clin Invest 83: 978, 1989

Wright SD, Rao PE, Van Voorhis WC, Craigmyle LS, Iida K, Talle MA, Westberg EF, Goldstein G, Silverstein SC: Identification of the C3bi receptor of human monocytes and macrophages by using monoclonal antibodies. Proc Natl Acad Sci USA 80: 5699, 1983

Wright SD, Jong MTC: Adhesion-promoting receptors on human macrophages recognize escherichia coli by binding to lipopolysaccharide. J Exp Med 164: 1876, 1986a

Wright SD, Meyer BC: Phorbol ester cause sequential activation and deactivation of complement receptors on polymorphonuclear leukocytes. J Immunol 136: 1759, 1986b

Wright SD, Reddy PA, Jong MTC, Erickson BW: C3bi receptor (complement receptor type 3) recognizes a region of complement protein C3 containing the sequence Arg-Gly-Asp. Proc Natl Acad Sci USA 84: 1965, 1987

Wright SD, Weitz JI, Huang AJ, Levin SM, Silverstein SC, Loika JD: Complement receptor type three (CD11b/CD18) of human polymorphonuclear leukocytes recognizes fibrinogen. Proc Natl Acad Sci USA 85: 7734, 1988

Wylie DE, Damsky CH, Buck CA: Studies on the function of cell surface glycoproteins 1. Use of antisera to surface membranes in the identification of membrane components relevant to cell-substrate adhesion. J Cell Biol 80: 385, 1979

Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332: 411, 1988

Yannariello-Brown J, Wewer U, Liotta L, Madri JA: Distribution of a 69 kD laminin-binding protein in aortic and microvascular endothelial cells: modulation during cell attachment, spreading, and migration. J Cell Biol 106: 1773, 1988

Yap HK, Sakai RS, Bahn L, Rappaport V, Woo KT, Ananthuraman V, Lim CH, Chiang GSC, Jordan SC: Anti-vascular endothelial cell antibodies in patients with IgA nephropathy: frequency and clinical significance. Clin Immunol Immunopathol 49: 450, 1988

Yasuda T, Gold HK, Fallon JT, Leinbach RC, Guerrero JL, Scudder LE, Kanke M, Shealy D, Ross MJ, Collen D, Coller BS: Monoclonal antibody against the platelet glycoprotein (GP) IIb/IIIa receptor prevents coronary artery reocclusion after reperfusion with recombinant tissue-type plasminogen activator in dogs. J Clin Invest 81: 1284, 1988

Yednock TA, Butcher EC, Stoolman LM, Rosen SD: Receptors involved in lymphocyte homing: relationship between a carbohydrate-binding receptor and the MEL-14 antigen. J Cell Biol 104: 725, 1987

Yu CL, Haskard DO, Cavender D, Johnson AR, Ziff M: Human gamma interferon increases the binding of T lymphocytes to endothelial cells. Clin Exp Immunol 62: 554, 1985

Yu CL, Haskard D, Cavender D, Ziff M: Effects of bacterial lipopolysaccharide on the binding of lymphocytes to endothelial cell monolayers. J Immunol 136: 1986

Zhou DFH, Ding JF, Picker LJ, Bargatze RF, Butcher EC, Goeddel DV: Molecular cloning and expression of Pgp-1. The mouse homolog of the human H-CAM (Hermes) lymphocyte homing receptor. J Immunol 143: 3390, 1989

Zimmerman GA, McIntyre TM, Prescott SM: Thrombin stimulates the adherence of neutrophils to human endothelial cells in vitro. J Clin Invest 76: 2235, 1985

Zimmerman GA, McIntyre TM: Neutrophil adherence to human endothelium in vitro occurs by CDw18 (Mol, MAC-1/LFA-1/GP 150,95) glycoprotein-dependent and -independent mechanisms. J Clin Invest 81: 531, 1988

Zimrin AB, Eisman R, Vilaire G, Schwartz E, Bennett JS, Poncz M: Structure of platelet glycoprotein IIIa. A common subunit for two different membrane receptors. J Clin Invest 81: 1470, 1988

Zinkernagel RM, Doherty PC: Restriction of in vitro T cell mediated cytotoxicity in lymphocyte choriomeningitis within a syngeneic or semiallogeneic system. Nature 248: 701, 1974

Zsebo KM, Yuschenkoff VN, Schiffer S, Chang D, McCall E, Dinarello CH, Brown MA, Attrack B, Bagby GC: Vascular endothelial cells and granulocytes: interleukin-1 stimulates release of G-CSF and GM-CSF. Blood 71: 99, 1988

Zweiman B, Moskowitz AR, Lisak RP: Quantitative assessment of the adherence of normal human blood mononuclear cells to vascular endothelial cell monolayers. Cell Immunol 68: 165, 1982

Zylstra S, Chen F, Ghosh SK, Repasky EA, Rao U, Takita H, Bankert RB: Membrane-associated glycoprotein (gp 160) identified on human lung tumors by a monoclonal antibody. Canc Res 46: 6446, 1986

APPENDIX: PUBLICATIONS ARISING FROM THIS THESIS

1. Faull RJ, Starr RJ, Russ GR: Vascular endothelial cell expression of adhesion molecules and HLA antigens in renal allografts. Transplantation Proceedings 20: 14, 1988
2. Faull RJ, Russ GR: Tubular expression of intercellular adhesion molecule -1 (ICAM-1) during renal allograft rejection. Transplantation 48: 226, 1989
3. Faull RJ, Russ GR: Adhesion of lymphocytes to stimulated vascular endothelial cells occurs via ICAM-1-dependent and ICAM-1-independent pathways. Transplantation Proceedings 22: 2099, 1990
4. Faull RJ, Russ GR: Very late antigen molecules in renal allografts. Transplantation Proceedings (in print)
5. Panegyres PK, Faull RJ, Russ GR, Appleton SL, Wangel AG, Blumbergs PC: Endothelial cell activation in vasculitis of peripheral nerve and skeletal muscle. Journal of Neurology, Neurosurgery, and Psychiatry (in print)
6. Faull RJ, Russ GR: A monoclonal antibody against the integrin β_1 chain identifies specific subgroups of the very late antigens (VLA) family of receptors. (manuscript in preparation)
7. Faull RJ, Russ GR: The monoclonal antibody PHM2 inhibits cell adhesion to fibronectin and recognises VLA-5. (manuscript in preparation)
8. Faull RJ, Russ GR: Comparison of the expression of β_1 integrins in the normal kidney and in rejecting renal allografts. (manuscript in preparation)